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THE BIOCHEMICAL EFFECTS OF SEX HORMONES. ACID AND ALKALINE PHOSPHATASE ACTIVITY, CALCIUM, AND PHOSPHORUS

KENNETH W. BUCHWALD AND LEONA HUDSON

From the Department of Biochemistry, State Institute for the Study of Malignant Diseases

BUFFALO, NEW YORK

THE RELATIONSHIP of the male hormone to carcinoma of the prostate has recently created widespread interest. Elimination of the male hormone from the body by castration or its counteraction through the administration of diethylstilbestrol affects the growth and activity of the carcinomatous prostate gland. Herger and Sauer (1943) have reviewed the literature on the effect of castration on the prostate gland and the clinical results following orchidectomy and treatment with diethylstilbestrol in patients with metastatic carcinoma of the prostate. A general improvement in the condition of the patient, relief from pain, and regression in size and softening of the prostate are some of the favorable clinical results obtained. The serum acid phosphatase activity immediately decreases and the serum alkaline phosphatase activity may or may not increase followed by a decrease. No changes are observed in the serum calcium and phosphorus.[1]

Kutscher and Wolbergs (1935) pointed out that of the human male genital organs the prostate showed the greatest acid phosphatase activity. Gutman, Sproul, and Gutman (1936) confirmed this observation and added that prostatic tissue which had undergone a carcinomatous change still retained this characteristically high acid phos-

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[1] Unpublished data from the Biochemical Department of the State Institute for the Study of Malignant Diseases.

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phatase activity. Even in metastatic carcinoma of the prostate the metastatic growths possessed the ability to produce large amounts of acid phosphatase. Gutman and Gutman (1938 a) further demonstrated that the prostate had a much higher acid phosphatase activity than any other tissue of the human body. When carcinoma of the prostate metastasized to the skeleton the acid phosphatase activity of the blood serum rose above normal. They also demonstrated that this serum acid phosphatase was identical with the acid phosphatase found in the prostate gland (Gutman and Gutman, 1938 b). Huggins and Hodges (1941) reported that bilateral orchidectomy or administration of diethylstilbestrol caused a decrease in serum acid phosphatase activity while testosterone propionate caused the serum acid phosphatase activity to increase in patients with metastatic carcinoma of the prostate. Within a few days after orchidectomy or the admintration of diethylstilbestrol the serum acid phosphatase activity was lowered and eventually approached the normal value. At the same time there was a temporary rise in the serum alkaline phosphatase activity which later decreased.

These observations have led us to study the biochemical process involved in this new method of treating carcinoma of the prostate. Since carcinoma of the prostate metastasizes to bone in a great majority of the cases, metabolic changes produced by diethylstilbestrol might be reflected in the calcium, phosphorus, and acid and alkaline phosphatase activity of the blood and tissues.

EXPERIMENTAL

Wistar albino rats, 125–135 days old at the beginning of the experiment, were used. Distribution of 63 rats was made according to litter, age, and weight as follows: (1) male and female controls, (2) males and females injected subcutaneously with 0.1 cc. of sesame oil, (3) males injected subcutaneously with 0.1 mg. of diethylstilbestrol in 0.1 cc. of sesame oil,[2] (4) females injected subcutaneously with 0.2 mg. of testosterone propionate in 0.1 cc. of sesame oil.[3] For a 28-day period the animals were injected daily except Sundays; double doses were given on Saturdays. The rats were given Purina fox chow and drinking water ad libitum. They were kept on wire screens throughout the experiment. All animals were weighed once a week.

At the end of the 28-day period of injections the animals were killed by bleeding from the carotid artery. The rats under a light ether anaesthesia were strapped on their backs to an operating board. The skin over the front of the neck was removed. The submaxillary glands and muscle layers were separated by blunt dissection to expose the trachea. The right common carotid artery was freed from the right vagus nerve and all surrounding tissue. A ligature of cotton thread was applied to the artery as far away from the

[2] Stilbestrol, 1 cc. size ampoules, manufactured by Sharpe and Dohme, Philadelphia, Pennsylvania.

[3] Neo-hombreol, 1 cc. size ampoules, each containing 25 mg. of testosterone propionate in peanut oil, Roche-organon, Inc., Roche Park, Nutley, New Jersey, diluted with sesame oil 1: 12.5.

heart as possible. Just caudad to this ligature a second ligature was applied to which was fastened a two-gram weight. The common carotid artery was then severed between the two ligatures. The operating board was inclined to an angle of 45 degrees with the rat's head down. The free end of the common carotid artery with the weight attached was placed in a test tube. A small nick was made in the artery close to the ligature with a pair of sharp pointed scissors. The administration of ether was then stopped so normal respiration would continue as long as possible. Usually 8–10 cc. of blood was obtained which was set aside to clot undisturbed.

As soon as the clot had formed the blood was centrifuged. The acid and alkaline phosphatase activities were determined immediately. The calcium was precipitated and allowed to stand overnight. The following day the phosphorus was determined on the supernatant fluid from the calcium precipitation. The femurs were removed, all adhering fat, muscle, and connective tissue were trimmed off, and after a thorough cleaning by rubbing with a piece of cloth they were placed in stoppered test tubes for weighing. Immediately after weighing, one femur was crushed and ground with successive portions of 2 cc. each of Dixon-Ringer's solution. Each portion of the extracting fluid was drained off into an Erlenmeyer flask before the next was added. With the last two portions of extracting fluid the crushed bone was washed into the flask. A total of 10 cc. of Dixon-Ringer's solution was used to extract the phosphatase from the femur. This extract to which was added a few drops of toluene was allowed to stand in the refrigerator for 48 hours in contact with the crushed bone. The extract was filtered through filter paper and the alkaline phosphatase activity determined on an aliquot portion. The other femur for ash determination was extracted in a Soxhlet extractor for two days with ethyl alcohol and one day with ether, then ashed to constant weight in an electric furnace at dull red heat. From the males the testicles were removed and weighed. From the females the uterus, oviduct, and ovaries were removed. After all the connective tissue and fat had been trimmed off, they were weighed as a unit.

To determine the calcium and phosphorus intake and output eight animals were housed in individual metal cages with wire bottoms of one-fourth inch mesh. The cages rested on the sides of metal trays three inches high. Between the cage and the bottom of the tray a wire screen of three-sixteenths inch mesh was placed to collect the feces but allow the wasted food to drop through. The wasted food was collected on coarse filter paper in the bottom of the tray. The daily intake and output of calcium and phosphorus was followed in (1) two males and two females receiving 0.1 cc. of sesame oil, (2) two males receiving 0.1 mg. of diethylstilbestrol in 0.1 cc. of sesame oil. (3) two females receiving 0.2 mg. of testosterone propionate on 0.1 cc. of sesame oil. Except for a four-day period for female rat 4 the feces were collected for a preliminary period of five days before any injections were made. At the same time each day except Sundays the rats were injected, weighed, and given a weighed amount of food. The food supplied was always in excess of the daily requirements. The unconsumed and wasted food was weighed to determine the food consumption for the past 24 hours. On Saturdays double doses were injected and a 48-hour supply of food was given the rats. The collection of feces on Mondays covered a 48-hour period. Distilled water was given the animals ad libitum. Each day a checker of food was set aside as a sample. At

the end of the experimental period the collected checkers were ground to form a composite sample which was analyzed for calcium and phosphorus.

Methods: The serum calcium was determined by the Clark-Collip (1925) modification of the Kramer-Tisdall method with the following variations: 1 cc. of serum and 3 cc. of water were used for the calcium precipitation and 0.005 N potassium permanganate was used for the titration. The phosphorus determination was made on the supernatant fluid from the calcium oxalate precipitation as suggested by Gunther and Greenberg (1929). The phosphatase activity was found by determining the amount of phenol liberated from disodium phenyl phosphate.[4] The alkaline phosphatase activity was determined at pH 9.0 according to the method of King and Armstrong (1934) using 0.1 cc. of serum plus 0.4 cc. of normal saline. The acid phosphatase activity was determined at pH 5.0 according to the procedure of Gutman and Gutman (1940). For the determination of alkaline phosphatase activity in the bone 0.1 cc. of the extract of the femur plus 0.4 cc. of normal saline was used. The collected feces were ignited in quartz dishes over Meeker burners and finally reduced to a white ash in an electric furnace at dull red heat. The ash was dissolved in 20 cc. of 1:3 hydrochloric acid and 3 cc. of nitric acid. Any pyrophosphate which might have formed during the ignition was converted to orthophosphate by the addition of the nitric acid. After extracting for 30 minutes on a boiling water bath the solution of the ash was transferred to a 100 cc. volumetric flask, made up to volume, and then filtered through ashless filter paper. Calcium and phosphorus were determined on aliquot portions of the filtrate. The volumetric method of McCrudden (1911) was used for the analysis of calcium. The phosphorus was determined by double precipitation and weighing as magnesium pyrophosphate according to Neumann as outlined by Hawk and Bergeim (1937).

DISCUSSION

Tables 1 and 2 summarize the results of injecting diethylstilbestrol into intact male rats and testosterone propionate into intact female rats. Columns 1, 2, and 3 give the means together with their probable errors of the control, sesame oil, and hormone injected rats. It was suspected that rats receiving sesame oil would show no effects of the injections and, therefore, this group rather than the noninjected group would be used as a control for the hormone injected rats. Accordingly, the groups of rats receiving sesame oil and hormones were kept equal in number, though in a few instances not enough blood was obtained from the animals to do all the analyses. Column 4 shows how many times the difference of the means of the control and oil injected rats exceeds the probable error of the difference of these means. Col-

[4] Disodium phenyl phosphate supplied through the courtesy of Mr. H. A. Pinkalla, Paul-Lewis Laboratories, Inc., Milwaukee, Wisconsin.

umn 5 shows how many times the difference of the means of the hormone injected and oil injected rats exceeds the probable error of the difference of these means. The values in Columns 4 and 5 had to be greater than three before differences could be considered significant. The weight difference is the body weight at the end of the experiment minus the body weight at the beginning. In Table 3 the intake and

TABLE 1. EFFECTS OF THE INJECTION OF DIETHYLSTILBESTROL ON MALE RATS

	1 Control	2 Oil	3 Diethylstilbestrol	4	5
Number of rats	9	12	12		
Weight difference, gm.	21 ±2.39	33 ±2.04	−53 ±3.82	3.82	19.8
Femurs, fresh weights, gm.					
1.	0.88±0.016	0.93±0.019	0.88±0.018	2.01	1.91
2.	0.87±0.019	0.90±0.020	0.89±0.018	1.09	0.37
Femur, weight of ash, mg.	402.7 ±8.96	423.2 ±7.94	419.4 ±8.71	1.77	0.32
Sex organs, weight, gm.	2.69±0.036	2.72±0.041	0.67±0.013	0.55	47.7
Serum					
Calcium, mg. %	11.25±0.17	10.98±0.16	10.77±0.15*	1.16	0.96
Phosphorus, mg. %	6.86±0.23	6.42±0.17	5.82±0.087*	1.54	3.14
Phosphatase, units per 100 cc.					
Acid	6.5 ±0.56	5.3 ±0.21	2.7 ±0.11*	2.01	10.96
Alkaline	66.3 ±3.23	75.3 ±2.81	72.3± 2.95*	2.10	0.74
Bone					
Alkaline phosphatase, units per gm.	7.9 ±0.21	8.9 ±0.43	5.6 ±0.34	2.09	6.02

* Analysis of serum from 11 rats.

output of calcium and phosphorus are shown for a preliminary five-day period and the 28-day experimental period. The intake of calcium and phosphorus was calculated from the analyses of the composite sample of the fox chow which was collected over the whole ex-

TABLE 2. EFFECTS OF THE INJECTION OF TESTOSTERONE PROPIONATE ON FEMALE RATS

	1 Control	2 Oil	3 Testosterone propionate	4	5
Number of rats	6	12	12		
Weight difference, gm.	16 ±1.83	17 ±1.28	37 ±1.91	0.45	8.69
Femurs, fresh weights, gm.					
1.	0.63±0.014	0.64±0.0058	0.72±0.027	0.66	2.90
2.	0.62±0.011	0.65±0.010	0.69±0.012	2.02	2.56
Femur, weight of ash, mg.	296.4 ±5.50	300.1 ±3.48	324.0 ±6.68	0.57	3.21
Sex organs, weight, gm.	0.67±0.018	0.67±0.030	0.45±0.015	0.00	6.55
Serum					
Calcium, mg. %	11.38±0.23	10.74±0.15	10.66±0.13*	2.33	0.42
Phosphorus, mg. %	5.85±0.21	6.21±0.13*	6.20±0.20*	1.46	0.042
Phosphatase, units per 100 cc.					
Acid	7.0 ±0.92	5.1 ±0.34*	5.2 ±0.26*	1.93	0.23
Alkaline	69.7 ±3.17	67.5 ±3.42*	82.7 ±2.19	0.47	3.74
Bone					
Alkaline phosphatase, units per gm.	9.5 ±0.37	9.4 ±0.20	10.3 ±0.43	0.17	1.90

* Analysis of serum from 11 rats.

perimental period. The calcium content of the sample was 1.34 per cent and the phosphorus 0.82 per cent.

The effect of both hormones on the growth of our rats is in agreement with reported observations: Richards and Kueter (1941) and Shay *et al.* (1941). The changes in body weights and atrophy of the sex organs, well established effects of both hormones demonstrate clearly that we used effective dosages for each hormone. Although

TABLE 3. EFFECTS OF THE INJECTION OF SEX HORMONES ON THE CALCIUM AND PHOSPHORUS METABOLISM

	Calcium intake mg. Ca	Calcium output mg. Ca	Calcium balance mg. Ca	Phosphorus intake mg. P	Phosphorus output mg. P	Phosphorus balance mg. P
	Oil Female 10, Litter 1					
Preliminary, 5 days	950	886	+64	583	594	− 11
Experimental, 28 days	5378	5449	−71	3299	3993	−694
	Female 4, Litter 3					
Preliminary, 5 days	664*	527*	+137*	407*	303*	+104*
Experimental, 28 days	4776	4682	+94	2923	2758	+165
	Testosterone Propionate Female 26, Litter 1					
Preliminary, 5 days	786	896	−110	478	506	−28
Experimental, 28 days	5374	5224	+151	3302	2977	+325
	Female 29, Litter 3					
Preliminary, 5 days	1142	928	+214	704	532	+172
Experimental, 28 days	5283**	4790**	+493**	3246**	3086**	+160**
	Oil Male 14, Litter 1					
Preliminary, 5 days	1178	1058	+120	722	577	+145
Experimental, 28 days	5898	5226	+672	3645	3310	+335
	Male 16, Litter 3					
Preliminary, 5 days	1301	1095	+206	795	590	+205
Experimental, 28 days	7421	6831	+590	4546	4068	+478
	Diethylsbilbestrol Male 37, Litter 1					
Preliminary, 5 days	1201	1065	+136	734	679	+55
Experimental, 28 days	3959	4342	−383	2425	3219	−794
	Male 40, Litter 3					
Preliminary, 5 days	1320	1024	+296	808	590	+218
Experimental, 28 days	3873	3867	+6	2730	2280	+450

* Period of 4 days.
** Period of 27 days.

not significant, the changes in the fresh and ashed weights of the femurs followed the changes in body weights. If the weights of the femurs are an index to skeleton weight, the changes which diethylstilbestrol and testosterone propionate produced in the body weights was not reflected to any marked degree in the bony tissue.

The serum calcium was unchanged by either testosterone propionate or diethylstilbestrol. The serum phosphorus was unaltered by testosterone propionate. However, the serum phosphorus of the males

injected with diethylstilbestrol was lower than either the control or sesame oil injected males. It is interesting to observe that the serum phosphorus of the control females was lower than the control males. Furthermore, males injected with diethylstilbestrol had a serum phosphorus level approximately that found in control females. One may speculate on the possibility that a reduction in serum acid phosphatase activity by diethylstilbestrol is further reflected in a reduction of the inorganic phosphorus of the blood serum.

Testosterone propionate produced no change in the serum acid phosphatase activity. On the other hand, diethylstilbestrol effected a very significant decrease in acid phosphatase activity of the blood serum in the intact male rats. This is precisely the action diethylstilbestrol has in patients with metastatic carcinoma of the prostate. Herger and Sauer (1943) reported that patients on diethylstilbestrol medication showed a reduction of serum acid phosphatase activity nearly to the normal value within two to three weeks. Gutman and Gutman (1939) found that the injection of testosterone propionate into the immature rhesus monkey increased the acid phosphatase content of the prostate gland to the level found in the adult monkey. They state that estradiol benzoate does not increase the acid phosphatase content. Huggins and Hodges (1941) state that androgen stimulates the production of serum acid phosphatase while estrogen suppresses its elaboration by metastatic carcinoma of the prostate. In our experiment diethylstilbestrol has suppressed the elaboration of acid phosphatase by normal glandular tissue. One cannot say definitely that diethylstilbestrol has acted directly on the prostatic tissue of the rat. It may be that other sex glands in the rat elaborate acid phosphatase for, unlike man, the rat's prostatic tissue has a low acid phosphatase content (Gutman and Gutman, 1938 a). Diethylstilbestrol was without influence on the serum alkaline phosphatase activity of the intact male rats. Testosterone propionate injected into intact female rats definitely increased the serum alkaline phosphatase activity. The increase in serum alkaline phosphatase activity is another indication that testosterone propionate is actively stimulating growth. In young children where the growth rate is high the serum alkaline phosphatase activity is slightly higher than in adults. Williams and Watson (1941) injected 5 mg. of testosterone into immature male rats and found that the alkaline phosphatase content of the femur increased. The increase persisted longer in the epiphysis than in the diaphysis. The increase of alkaline phosphatase activity of the bone which we obtained was not significant. However, this same increase together with the increase in serum alkaline phosphatase activity may be reflections of the stimulated body growth. In the males injected with diethylstilbestrol the alkaline phosphatase activity of the femurs decreased considerably. Since it is agreed that the alkaline phosphatase activity is a measure of the osteogenic ability of bone it seems

reasonable to believe that the lack of healing of bony metastases of carcinoma of the prostate may be due to the depressing action of diethylstilbestrol on alkaline phosphatase activity in bone.

Preliminary studies were designed to indicate any changes in the excretion of calcium and phosphorus. In determining the calcium and phosphorus output only the feces were analyzed. Since the fecal output of calcium is about 90 per cent of the total output, changes in the

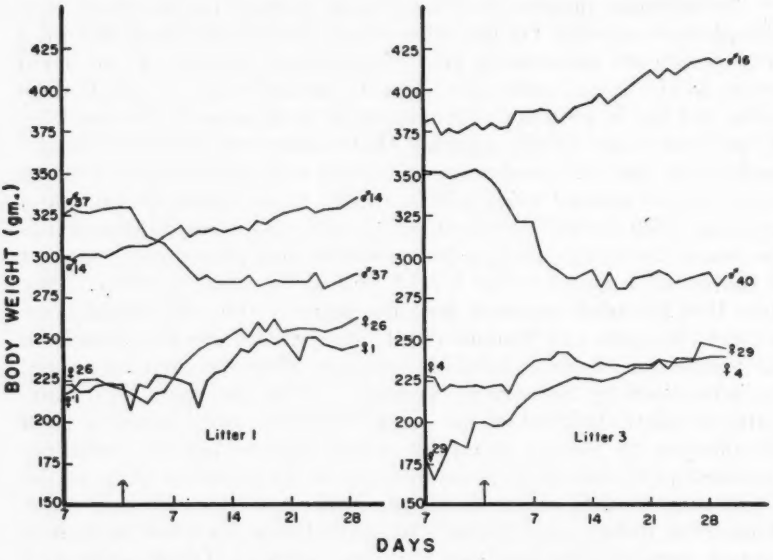

FIG. 1. Daily body weights of animals on metabolism experiment. Females 1 and 4, males 14 and 16, injected with 0.1 cc. of sesame oil. Females 26 and 29 injected with 0.2 mg. of testosterone propionate in 0.1 cc. of sesame oil. Males 37 and 40 injected with 0.1 mg. of diethylstilbestrol in 0.1 cc. of sesame oil. Arrow indicates day injections were started.

calcium metabolism should be detected by analysis of the feces only. To avoid the complication of collecting urine the phosphorus output was followed in the feces although only one-third of the total output of phosphorus is by way of the intestinal tract (Meyers and Fine, 1916). Throughout the experiment the phosphorus output rose or fell simultaneously with that of the calcium.

In Figure 1 the daily body weights for the animals on the metabolism experiment are shown. These changes in weight were characteristic of the other animals in the respective groups and served as criteria that the hormones were taking effect.

During the preliminary period all animals except females 1 and 26 were in positive balance for calcium and phosphorus. During the 28-

day experimental period all female rats except female 1 were in positive calcium balance. The intake of calcium and phosphorus for female 26 was the same as for control female 1 during the period of injections. The control males 14 and 16 maintained positive calcium and phosphorus balances throughout the 28 days of the experiment and showed a normal growth rate. During the period of diethylstilbestrol injections, male 37 showed a very definite deficit of both calcium and phosphorus. Thus it would appear that diethylstilbestrol and testosterone propionate had no specific influence on the fecal output of either calcium or phosphorus. It was concluded that more elaborate metabolic studies were not justified.

It should be pointed out that in this experiment normal rats were used. If conditions more closely resembling those found in humans with disseminated carcinoma of the prostate could be established the results might be more enlightening. The changes produced in the sex organs showed that sufficient doses of the hormones had been administered. No indication of a disturbance of calcium or phosphorus metabolism was shown. The decrease in the serum acid phosphatase activity produced by diethylstilbestrol is in agreement with the findings in metastatic carcinoma of the prostate in humans. The significance of the decrease in the alkaline phosphatase activity of the femurs is difficult to understand, all the more so since the fresh and ashed weights of the femurs showed no changes. It may, however, bear some relationship to the difficulty experienced in the healing of bone metastases in carcinoma of the prostate. Still further changes may be produced by removal of the testicles and ovaries so that the injected sex hormone might act without the presence of its antagonist. Experiments of this nature are now being completed.

SUMMARY

Daily subcutaneous injections of 0.1 mg. of diethylstilbestrol and 0.2 mg. of testosterone propionate were given mature male and female rats, respectively, for 28-day periods. There was no change in the serum calcium nor the output of calcium and phosphorus in the feces. The serum phosphorus was not influenced by the testosterone propionate, but diethylstilbestrol produced an apparent decrease. Diethylstilbestrol also produced a decrease in the acid phosphatase activity of the blood serum and the alkaline phosphatase activity of the femurs but had no influence on the alkaline phosphatase activity of the blood serum. The decrease in the alkaline phosphatase activity of the femurs may be related to the difficulty observed in the healing of bone metastases in carcinoma of the prostate. Testosterone propionate produced an increase in alkaline phosphatase activity of the serum without influencing the acid phosphatase activity of the serum or the alkaline phosphatase activity of the femurs.

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EFFECT OF SOME ANDROGENIC STEROIDS ON THE ADRENAL CORTEX OF HYPOPHYSECTOMIZED RATS[1]

SAMUEL L. LEONARD

From the Department of Zoology, Cornell University

ITHACA, NEW YORK

MAINTENANCE and stimulation of the adrenal cortex of hypophysectomized animals by hormones from sources other than the pituitary gland have been reported. Testosterone propionate decreased the rate of adrenal cortical atrophy which normally follows hypophysectomy in the young male rat (Cutuly, Cutuly, and McCullagh, 1938), (Leonard, 1942), (Leatham).[2] Thyroxin, insulin and non-hormonal toxic agents stimulated the adrenal cortex of hypophysectomized pigeons (Miller and Riddle, 1942) and the gonadotrophic principle from pregnant mare serum stimulated the adrenals of hypophysectomized ground squirrels (Zalesky, Wells, and Overholser, 1942).

In view of these findings, the effects of androgens on the adrenals of hypophysectomized rats were investigated. This report is concerned with (a) the effects of different dosages of testosterone propionate and other androgens on the maintenance of adrenal weight in hypophysectomized rats, and (b) the nature of this stimulation of the adrenal cortical cells.

METHODS

Long-Evans rats, used in these experiments, were fed an adequate diet supplemented with a 10% glucose solution. Injections, begun on the day of hypophysectomy (except in experiments on delayed treatment), were continued daily for 10 days, and autopsies were performed the day following the last injection. Observations were made on body weights and weights of the dissected adrenals, thyroids, testes, and in some cases, the ventral lobe of the prostate. The adrenal glands were prepared for routine histological examination and a few were stained for lipoid content according to the method of Gersh (1939). In some experiments, the testes also were studied histologically. No data are included on rats which had hypophyseal remnants in the sellae turcicae as determined by histological examination.

EXPERIMENTAL

In order to determine an adequate dosage of androgen to slow significantly the atrophy of the adrenal cortex which follows hy-

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[2] J. Leatham, personal communication.

pophysectomy, four litters of young male rats were injected with different dosages of testosterone propionate[3] for a period of 10 days, beginning on the day of the operation. Uninjected hypophysectomized rats from each litter served as controls. The results in Table 1 (A) show that the stimulation of the adrenals was directly proportional

TABLE 1. TESTOSTERONE STIMULATION OF THE ADRENAL CORTEX OF HYPOPHYSECTOMIZED RATS

Exper.	Age	Weight	Dosage	Adrenal weight		Diff. in adrenal wt.	Diff. in testes wt.
				Exper.	Control		
		gms.	mg.	mg.	mg.	%	%
A. Young males, graded dosages.							
C109	28	68	.67	7.6 (3)*	6.8 (3)	12	87
C106	39	114	1.38	11.1 (3)	8.5 (3)	31	174
C104	27	88	2.75	9.7 (3)	6.5 (3)	49	257
C 98	32	77	4.26	14.4 (4)	9.0 (3)	60	155
B. Young males, postponed treatment for 10 days.							
C107	46	130	2.50	8.6 (3)	7.0 (2)	23	18
C108	36	99	2.50	8.3 (4)	6.8 (2)	22	40
C 99	31	87	7.50	8.3 (3)	6.3 (3)	32	56
C128	29	61	12.50	6.6 (3)	5.7 (2)	16	31
C150†	49	120	15.00	7.6 (4)	7.2 (3)	6	45
C. Adult males							
C 89	61	207	12.50	18.4 (4)	12.5 (4)	47	162
C 85	75	205	12.50	17.8 (3)	14.8 (4)	20	60
	—	161	12.50	17.6 (1)	12.9 (1)	36	91
C 90	75	198	25.00	19.6 (2)	14.6 (2)	34	28
C124	68	209	25.00	18.6 (4)	15.4 (3)	21	29
D. Young females							
C125	31	60	11.25	7.2 (3)	7.9 (4)	−9	—
C114	—	60	12.50	13.0 (3)	10.3 (4)	26	—
C115	32	77	12.50	12.8 (2)	8.2 (3)	56	—
C126	37	83	12.50	10.4 (3)	8.5 (2)	22	—
C132	32	81	12.50	11.5 (2)	7.6 (2)	51	—

* Numbers in ( ) refer to number of animals.
† Treatment postponed 20 days, injections for 12 days thereafter.

to the size of the dosage. The excess in the weight of the adrenals of the injected rats is expressed as a percentage of the adrenal weight of the operated control animals. With lower dosages of hormone, the percentage was smaller. A difference of 20 percent as a minimum was assumed to be significant in this, and in subsequent, experiments because below this level the individual adrenal weights were not consistently heavier than those of the controls. With the lowest dosage of

[3] Acknowledgment is made to Dr. E. Oppenheimer of the Ciba Pharmaceutical Co., Summit, N. J., for his generous supply of all the hormones used in these experiments.

hormone used (.67 mg.), the difference in adrenal weights was below 20 percent.

In almost all litters, the treated rats maintained their body weights better, and appeared healthier and more active than did their respective controls. Partial maintenance of the testis weight was observed in the treated animals although at the lowest dosage of hormone used, the percentage difference in testis weight was noticeably less. The thyroid glands were not affected by testosterone propionate or by the other steroids used in subsequent experiments.

Postponed treatment. After adrenal atrophy had progressed for 10 days following hypophysectomy, testosterone propionate was injected to determine if the cortical cells would respond to delayed treatment. The results obtained from four litters showed that the adrenals of these rats were 16–32 percent heavier than those of the untreated litter-mate controls, Table 1 (B). A small but definite stimulation of the testes was also noted.

In another litter, treatment was postponed for 20 days. The differences in adrenal weights were not significant, but the testes of the enjected animals were slightly but uniformly heavier than those of ithe controls, Table 1 (B); experiment C 150.

Adult males and females. In order to determine if atrophy of the adrenal cortex of adult males could be delayed by testosterone propionate, 5 litters of young adult male rats were hypophysectomized and treated with large dosages of the hormone. The adrenal glands of these animals also were stimulated, but not to the same extent as when younger males were used, Table 1 (C). The greatest adrenal stimulation was observed in the youngest group of rats in this series. Young female rats were also hypophysectomized and injected with testosterone propionate to determine if there was a sex difference in the response. In 4 of the 5 litters used, the adrenal glands of the injected rats did not involute so rapidly as did those of the hypophysectomized controls, Table 1 (D). The uteri of the injected animals were stimulated markedly, but the ovaries were unaffected.

Effects of other steroid hormones. A limited number of other steroid hormones were available for these studies. Progesterone did not stimulate the adrenal cortex in hypophysectomized young male rats (3 injected animals) in dosages as high as 30 mg. given over a period of 10 days. The testes of these rats, however, were 190–200 percent heavier than those of the uninjected hypophysectomized controls. Desoxycorticosterone acetate administered to young hypophysectomized male rats in doses of 1 mg. daily for 10 days, failed to stimulate either adrenals, testes, or the ventral lobe of the prostate. The average weight of the adrenals of 3 injected rats was 8.3 mg. and of 3 control rats, 8.6 mg.

Four physiologically active androgenic steroids were tested for their ability to maintain the adrenal weights in young hypophysecto-

mized male rats. Doses of 1 mg. were given daily for 10 days, beginning on the day of operation. It was found that androstanediol-3-α-17-trans was the most effective of this group of hormones in maintaining adrenal weights. Δ^5 androstenediol-3-B-17-trans stimulated the adrenals to a limited extent, while androstenedione and trans-dehydroandrosterone failed to maintain the adrenal weights significantly (Table 2).

A positive correlation between the maintenance of adrenal and testis weight was noted in the animals receiving these androgenic steroids. The testes of the rats treated with androstenediol and androstanediol revealed a much higher degree of spermatogenic activity than did the testes of the respective controls. On the other hand, the

TABLE 2. STIMULATION OF THE ADRENAL CORTEX OF HYPOPHYSECTOMIZED RATS BY CERTAIN STEROIDS

Exper.	Hormones*	Adrenal Weight		Diff. in Adrenal Wt.	Diff. in Testes Wt.
		Exper.	Control		
		mg.	mg.	%	%
C119	Δ^5-Androstenediol-3-β-17 trans	9.2 (3)†	7.5 (2)	23	68
C123	Δ^5-Androstenediol-3-β-17 trans	9.8 (3)	8.2 (2)	20	162
C120	Androstenedione	7.0 (3)	7.5 (3)	−7	24
C151	Androstenedione	10.4 (3)	9.6 (2)	8	28
C118	Trans-dehydroandrosterone	8.1 (3)	7.5 (2)	8	29
C122	Androstanediol-3-α-17-trans	10.1 (3)	5.8 (2)	74	164
C131	Androstanediol-3-α-17-trans	16.6 (4)	9.1 (3)	82	154

* Dosages, 10 mg. in a period of 10 days.
† Numbers in ( ) refer to number of animals.

testes of the rats treated with androstenedione and trans-dehydroandrosterone, although slightly heavier, were indistinguishable from their controls when examined microscopically. A similar relationship was observed between maintenance of the adrenals and the degree of stimulation of the ventral lobe of the prostate. Androstenedione and trans-dehydroandrosterone, although incapable of maintaining adrenal weight, increased the weight of the prostate glands by 236–281 percent.

OBSERVATIONS ON THE NATURE OF THE CORTICAL STIMULATION

The adrenal cortices of the injected hypophysectomized rats resembled those of normal rats with intact pituitaries and were in great contrast to the atrophied glands of the controls. The androgens affected mainly the zona fasiculata and zona reticularis. The cells in these regions were plump and rich in stainable cytoplasm, contained few vacuoles, and possessed clear rounded nuclei as a result of the hormone injected. On the other hand, cells from similar regions in the control glands were shrunken, heavily vacuolated, and poor in stainable cytoplasm; many of the nuclei were deeply stained and irregularly shaped. In the cortices of androgen-treated rats, however, slight

degenerative changes had occurred and in no case was the histological condition normal.

In several instances, glands from injected and control animals were osmicated according to the method of Gersh (1939). The osmophilic globules in the cells of the zona fasiculata and zona reticularis of the treated rats were numerous, small, uniformly round, and uniformly dispersed. In contrast, the osmophilic material in similar regions of the control gland was in the form of large droplets, unequal in size, and unevenly dispersed in the cells. Clumping, coalescing, and evidence of dissolution of the lipoid material also were noted.

These morphological observations of the effects of androgens were made from experiments in which the adrenals were at least 20 percent heavier than their respective controls. Similar effects of the androgens upon the cortical cells were seen in some experiments in which the weight of the adrenal was not significantly maintained. For example, it was possible to distinguish microscopically the adrenals from injected rats in experiments C 125 and C 150, Table 1, in which the weights of the adrenals indicated no stimulation. The identification of the glands from rats treated with androstenedione and transdehydroandrosterone was not accomplished in every case.

To determine if testosterone propionate was maintaining adrenal weight in hypophysectomized rats by increasing the number of cells in the cortex, an experiment was devised to count the mitotic figures in the glands. A group of hypophysectomized male rats were treated with testosterone propionate and on the 10th day, colchicine in doses of 1 gamma per gram of body weight was given to both the experimental and litter-mate control animals. The adrenals were removed 9 hours later, weighed, fixed, and sectioned serially at 8 micra. Counts of the number of mitotic figures were made in the first section in which medullary cells appeared and in every 10th section which had medullary cells. Since the medullary portion of the adrenal is not influenced by hypophysectomy (Cutuly, 1936), a fairly equal number of representative sections (8–10) were examined from the glands of the injected and control rats. The total counts for each adrenal and the respective weights are given in Table 3.

By comparing the representative counts per gland of the treated rats with those of the respective controls, it was noted that in only 4 of the 9 injected rats were there a greater number of mitotic figures. Similarly, in 5 of the 7 controls, the number of mitotic figures exceeded those found in the injected rats. Increased mitosis evidently was not a factor in maintaining adrenal weight in hypophysectomized rats treated with testosterone propionate.

DISCUSSION

Testosterone propionate, androstanediol, and androstenediol were able to stimulate the adrenal cortex of hypophysectomized rats to

the extent that the adrenals did not lose weight so rapidly as those of the uninjected controls. Androstenedione, trans-dehydroandrosterone, progesterone, and desoxycorticosterone were ineffective. Selye (1940) has reported that progesterone did not stimulate the adrenals of hypophysectomized female rats. This effect on the weight of the adrenals was observed best when adequate amounts of androgens were given for periods of only 10 days and treatment was begun on the day of operation. When treatment was postponed for 10 days, a slight, but positive, stimulation occurred; after 20 days, the weight of the

TABLE 3. TESTOSTERONE EFFECT ON RATE OF MITOSIS IN ADRENAL CORTEX OF HYPOPHYSECTOMIZED RATS

Exper.	Injected		Control		Diff. in adrenal wt.
	Weight of adrenals	No. of mitoses per gland	Weight of adrenals	No. of mitoses per gland	
	mg.		mg.		%
C111	7.0	30	6.1	25	15.0
C 113	13.5	133	11.0	92	18
	12.5	37			
	14.5	52	8.2	121	77
	15.0	99	8.5	210	
C136	9.5	38	8.2	93	
	12.0	38	7.0	57	34
	10.2	72	8.3	81	
	10.3	73			

adrenals of the injected and of the control rats did not differ. No experiments were made in which injections, begun on the day of operation, were continued longer than 10 days. Data from Noble's (1939) experiments (in which he injected progesterone, or pregnant mare serum, both alone and with testosterone propionate, into hypophysectomized rats for periods of 20 days) showed that the adrenals of testosterone-propionate-treated rats were always at least 20 percent heavier than the controls. This observation was not mentioned in his paper.

The adrenals of hypophysectomized adult male and female rats were also stimulated by testosterone propionate. The percentage differences in adrenal weights, when compared with the appropriate controls, were not so great as when young male rats were employed. It did not appear to be a matter of dosage, because adequate amounts of hormone, calculated on the basis of body weight of young male rats, were given in each case.

The androgens which most readily stimulated the adrenal cortex, also stimulated the testes and the ventral lobe of the prostate to the greatest degree. This was observed not only as an increased weight of the testes and prostate but also as an increased spermatogenic activity

in the testes. Androstenedione and trans-dehydroandrosterone were found to be weak in androgenic effects and ineffective in stimulating adrenal weight. Pregnenolone, which maintained spermatogenesis in hypophysectomized rats without stimulation of the seminal vesicles, was incapable of stimulating adrenal weight (Leatham and Brent, 1943). Correlation of all of the physiological effects of these androgenic substances requires further study.

The increased weight of the adrenals induced by these androgens seemed to be the result of maintenance of cell size in the cortex. The width of the cortex was obviously greater in those animals which were adequately treated. The cells of the zona fasiculata and zona reticularis resembled those in the adrenals of normal rats. The distribution and nature of the lipoid material in the cortex of treated animals resembled that in the normal gland. Since no increase was observed in the rate of mitosis in the cortex of treated rats, it appeared that an increase in the number of cells could not account for the heavier adrenals. Testosterone propionate increased mitosis in the adrenal cortex of normal rats (Nathanson and Brues, 1941) but in view of the above experiments, apparently this does not occur in the absence of the hypophysis.

Although weighing the adrenals offered a simple test for determining the effects of androgens on the cortex, it was not the most sensitive test. In several experiments, where the differences in weight were less than 20 percent, or even negligible, histological examinations of the glands readily permitted a separation of the glands of injected rats from those of the controls.

In hypophysectomized birds, stimulation of the adrenal cortex by non-pituitary substances, i.e., thyroxin, insulin and formaldehyde, induced adrenal growth exceeding that of normal controls, repaired the atrophic changes, and increased cell division in the cortex. Zalesky and his co-workers (1941) found that gonadotrophic hormone from pregnant mare serum, prospermin (an extract of normal urine of men), and the chorionic hormone prevented atrophy and stimulated the cortex in hypophysectomized ground squirrels. They attributed their results to adrenocorticotrophic substances contaminating the extracts. Testosterone, on the other hand, did not stimulate the cortex of these animals. Corticotrophic activity was reported in extracts of pregnant mare serum when tested on hypophysectomized rats (Golla and Reiss, 1942). Boiling the extract destoyed only the gonadotrophic activity. In view of these results, it may be that other substances independent of the pituitary can stimulate the adrenal cortex to a considerable degree.

SUMMARY

Testosterone propionate, androstanediol-3-α-17-trans, and androstenediol-3-B-17-trans decreased the rate of atrophy of the adrenal

cortex which normally follows hypophysectomy in young male rats. These results were obtained when adequate dosages of these androgens were injected, beginning on the day of operation and continued for 10 days. Similar results were obtained in adult male and in young female rats. When treatment was postponed for 10 days, less stimulation was noted; after 20 days, the weights of the adrenals were not increased.

Androstenedione, trans-dehydroandrosterone, progesterone, and desoxycorticosterone were ineffective.

In general, the ability of an androgen to stimulate the adrenal cortex was correlated with its ability to stimulate the testes and ventral lobe of the prostate.

Histological evidence of stimulation of the cortical cells was noted in some experiments where the weights of the adrenals were not significantly affected by androgens.

The partial maintenance of adrenal weight in these experiments was attributed to maintenance of cell size and not to an increase in the number of cells in the cortex.

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THE EFFECT OF PURE ADRENOCORTICOTROPIC HORMONE ON THE WORK PERFORMANCE OF HYPOPHYSECTOMIZED RATS

DWIGHT J. INGLE, CHOH HAO LI
AND HERBERT M. EVANS

From the Upjohn Research Laboratories, Kalamazoo, Michigan and The Institute of Experimental Biology, University of California,
BERKELEY, CALIFORNIA

CERTAIN CHANGES from normal in hypophysectomized animals are due to the absence of the adrenocorticotropic principle of the anterior pituitary and the consequent hypofunction of the adrenal cortex. The metabolic inadequacies of hypophysectomized rats are reflected by a greatly decreased capacity for work. Treatment of the hypophysectomized rat with either a partially purified adrenocorticotropic fraction of anterior pituitary extracts (Ingle, Moon and Evans, 1938) or with adrenal cortical extracts (Ingle, 1938a) has been shown to partially restore the capacity for work.

Li, Simpson and Evans (1942) have described the isolation of the adrenocorticotropic hormone in a form which meets biologic and physico-chemical criteria for homogeneity (Li, Evans and Simpson, 1943). The object of this study was to determine the effect of this principle, free from contamination by other pituitary hormones, on the ability of hypophysectomized rats to work. Positive results were obtained.

METHODS

Male rats of the Sprague-Dawley strain having an initial weight of 180 grams were used in these experiments. The diet was Purina Dog Chow. Hypophysectomies were carried out by the procedure of Ingle and Griffith (1942). The animals were maintained at a temperature of 25 to 27 degrees C. prior to and following the operation. The adrenocorticotropic hormone was prepared by the procedure described by Li, Evans and Simpson (1943). It was administered thrice daily to all of the treated animals in amounts of 0.5 mgm. per injection.

In the work tests, the animals were anesthetized with phenobarbital sodium. The gastrocnemius muscle was weighted with 100 grams and was made to contract three times per second by direct faradic stimulation. The amounts of work were recorded automatically. Stimulation was continued until the muscle lost responsiveness. A detailed description of the apparatus

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and methods has been published (Ingle, 1944). Following the beginning of stimulation each rat received 5 cc. of 0.9 per cent sodium chloride solution by subcutaneous injection thrice daily. The working animals were enclosed in a cabinet having a constant temperature of 28 degrees C. during the work test.

The analyses of glucose in tail blood were carried out by the method of Miller and Van Slyke (1936).

At the completion of the tests necropsies were performed in order to determine the completeness of hypophysectomy and to obtain weights of organs.

EXPERIMENTS AND RESULTS

In experiment 1, (Table 1) the work tests were begun fourteen days after hypophysectomy. Five rats were treated by thrice daily

RECORDS OF HYPOPHYSECTOMIZED MALE RATS (INITIAL WEIGHT 180 GRAMS) TREATED FOR FOURTEEN DAYS WITH PURE ADRENOCORTICOTROPIC HORMONE (0.5 MGM. 3 TIMES DAILY) AND THEN SUBJECTED TO THE WORK TEST

Rat	Mgm. daily dose	Final body wt.	Initial r.p.m.*	Total work**	Initial blood sugar mgm./% (fed)	Wt. mgm. adrenals	Wt. mgm. thymus	Wt. mgm. gonads
1	1.5	131	20	9074	109.5	53.5	23.0	644
2	1.5	132	20	17808	84.0	50.0	22.0	533
3	1.5	140	21	19312	87.0	53.5	39.0	632
4	0.0	150	8	1942	99.0	13.0	154.0	394
5	0.0	158	11	1411	94.5	14.0	253.0	422
6	0.0	154	8	2663	97.5	14.0	211.0	376
7	0.0	159	11	4808	93.0	12.0	237.0	897
8	0.0	160	12	4929	99.0	9.0	233.0	540
9	0.0	146	10	4923	97.5	15.0	179.0	491
10	1.5	141	21	54262	93.0	106.0	24.0	585
11	1.5	128	20	22535	69.0	73.0	27.0	1140

* Work is expressed in terms of recorder revolutions. Each revolution is equivalent to approximately 400 gram centimeters of work. r.p.m. indicates the number of revolutions per minute at the beginning of stimulation.

** The average value for total work by normal rats is approximately 100,000 recorder revolutions.

injections of adrenocorticotropic hormone in the amount of 0.5 mgm. per injection. Six rats were kept without treatment as controls. Rats 1, 2 and 3 continued to receive the hormone by intraperitoneal injection following the beginning of the work test. It was noted that after work had been continued for twenty-four hours or longer each intraperitoneal injection of the hormone was followed by a sharp but usually temporary decrease in the height of muscular contraction. Consequently, rats 10 and 11 were treated by intraperitoneal injection of the hormone up to the time of beginning the work test and were treated by subcutaneous injections of the hormone during the work test. No reaction to the injections was noted in these two animals.

Although the losses of weight were much greater in the treated group the initial heights of muscular contraction and the total amounts of work performed were much greater than among the un-

treated animals. The untreated rats all lost muscular responsiveness within twelve to eighteen hours. Rats 1, 2 and 3 (treated) all lost muscular responsiveness within twenty-four to thirty-six hours. Rat 10, worked for one hundred twenty hours and rat 11, worked for ninety-six hours. The terminal blood-sugar value for rat 10, was 24 mgm. per cent. At necropsy the adrenal glands of the untreated rats were found to be atropic whereas those of the treated rats showed marked hypertrophy. The thymus glands of the treated rats were atropic and the testicles of all the rats, treated and untreated, were atropic.

The values for blood glucose in samples taken just prior to the beginning of the work test were all within the limits characteristic of normal fed animals.

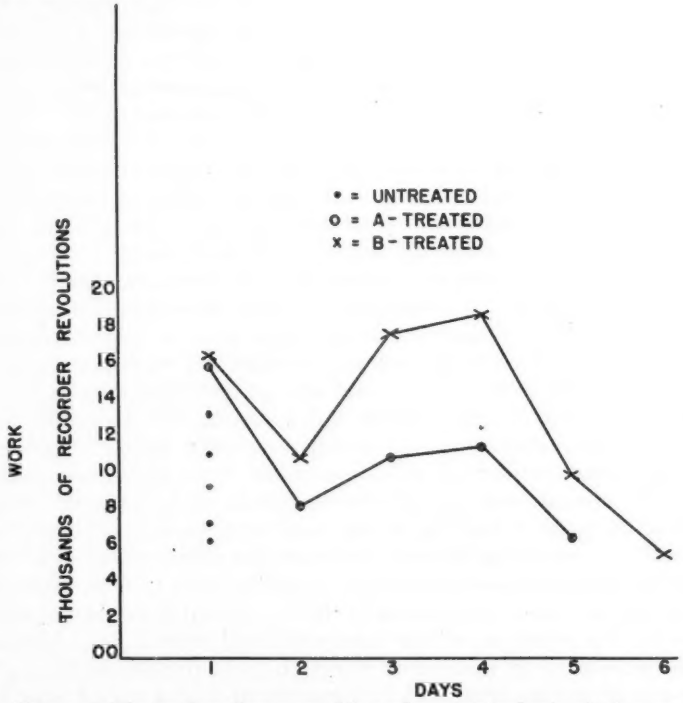

FIGURE 1. The work performance of hypophysectomized rats which were subjected to the work test immediately following hypophysectomy.

In experiment 2, (Figure 1) the rats were subjected to the work test immediately following hypophysectomy. The five untreated rats each showed a normal initial rate of work but each lost muscular responsiveness within eighteen hours after the beginning of stimulation. Two additional animals, rats A and B, were treated by the sub-

cutaneous injection of 0.5 mgm. of adrenocorticotropic hormone thrice daily. Rat A, worked for one hundred sixteen hours and at necropsy the body weight was 114 grams and the weight of the adrenal glands was 58 mgm. The terminal value for blood sugar was 20 mgm. per cent. Rat B, worked for one hundred forty-four hours and at necropsy the body-weight was 109 grams and the weight of the adrenal glands was 60 mgm. The terminal value for blood sugar was 18 mgm. per cent.

DISCUSSION

The results of these experiments provide additional evidence that the adrenocorticotropic hormone stimulates the adrenal cortex to greater functional activity as well as causing this "target organ" to hypertrophy.

The single dosage level tested in these experiments was known from previous studies (Li, Evans and Simpson, 1943) to cause a marked hypertrophy of the adrenal cortex in hypophysectomized rats. The duration of treatment (fourteen days) in Experiment 1, was selected to allow time for atrophy of the adrenal cortices of the untreated hypophysectomized animals and time for maximum cortical hypertrophy in the treated animals. Although the effect of the hormone upon work performance was very striking it is probable that a procedure of treatment might be found which would give a still greater restoration of the ability to sustain work. For example, the fact that the treated animals lost weight more rapidly than the untreated animals and that the adrenal cortices were greatly hypertrophied is evidence that a state of hypercortico-adrenalism existed prior to the beginning of the work test. Intact rats of 180 grams have adrenal glands averaging 27 mgm. weight but following the beginning of the work test they double in size within five days. (Ingle, 1938b). In order to duplicate physiological conditions it would be necessary to give only enough adrenocorticotropic hormone to prevent cortical atrophy until the beginning of the work test whereupon the dosage should be increased sufficiently to cause the same extent of cortical hypertrophy which occurs in intact working rats. It is possible that the amounts of work performed by Rats 1, 2 and 3 would have been greater if the reactions to the intraperitoneal injections of hormone had been avoided by making all injections subcutaneously during the work test as in rats 10 and 11 in Experiment 1 and rats A and B in Experiment 2.

Since the experimental animals were without food during the work test and were then dependent upon their own tissues as a source of energy it is obvious that the hypophysectomized rat was handicapped by its loss of weight in such a test of endurance. It is possible that the maintenance of a normal caloric intake and of body-weight by the force-feeding of hypophysectomized rats until the beginning of the

work test would result in a more nearly normal performance by the treated animals. It is unlikely that the performance of the hypophysectomized rat could be made normal by treatment with adrenocorticotrophic hormone alone since these animals have other metabolic deficiencies due to absence of other pituitary hormones.

It is of interest to note in Experiment 2, that the ability of the rat to work is decreased below normal within a few hours following hypophysectomy and prior to the beginning of cortical atrophy.

The total amounts of work performed by hypophysectomized rats treated with adrenocorticotropic hormone in these experiments was greater than that noted in earlier experiments on hypophysectomized rats treated with either a partially purified preparation of this hormone or with adrenal cortical extracts (Ingle, Moon and Evans, 1938). There were some differences in the experimental conditions of this and earlier experiments which may be of importance but it is also probable that the replacement therapy tested in the present study was more effective than the therapy used in the earlier studies.

SUMMARY

Two experiments were carried out to test the effect of pure adrenocorticotropic hormone upon the ability of hypophysectomized male rats to work. All of the treated rats received 0.5 mgm. of the hormone thrice daily following hypophysectomy. In Experiment 1, five treated rats were compared to six untreated rats after a postoperative period of fourteen days. The effect of the hormone was to cause an acceleration in loss of weight, a marked adrenal cortical hypertrophy instead of atrophy, thymus atrophy and a marked increase in the amount of work over the very poor performance of untreated animals. In Experiment 2, all animals were subjected to the work test immediately following hypophysectomy. The five untreated animals showed very poor performance as there was a complete loss of muscular responsiveness within eighteen hours. The two treated animals showed a greatly improved although subnormal performance and required one hundred sixteen and one hundred forty-four hours for complete fatigue.

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SENSITIVITY OF THE REPRODUCTIVE SYSTEM OF HYPOPHYSECTOMIZED 40 DAY MALE RATS TO GONADOTROPIC SUBSTANCES[1]

MIRIAM E. SIMPSON, CHOH HAO LI
AND HERBERT M. EVANS
From the Institute of Experimental Biology
University of California
BERKELEY, CALIFORNIA

IN THE purification of adrenocorticotropic hormone it was found that the standard test animal used, the male rat, when injected with pituitary fractions from the time of hypophysectomy, at 40–43 days of age, was extremely sensitive to the presence of pituitary interstitial cell stimulating hormone (ICSH). When traces of ICSH were present in pituitary fractions, the degree of differentiation of the testicular tubule at the time of operation was maintained, and sometimes even further differentiation of the testis occurred during the fifteen day injection period. It therefore seemed desirable to determine the sensitivity of this test animal to pure ICSH, as well as to various other gonadotropic agents, such as the hormones in pregnant mare serum and in human pregnancy urine.

Greep and Fevold (1937), reported the maintenance of spermatogenesis when injection was instituted immediately after hypophysectomy, and the re-establishment of spermatozoa after postoperative regression of the testes, as a result of injecting the "LH" fraction, admittedly not yet completely purified. Greep, van Dyke and Chow in 1942, reported "gross enlargement of the testes and secondary organs" and "stimulation of the seminiferous tubules and the interstitial cells" of immature rats, hypophysectomized at 21 days of age, and injected with pure pig ICSH from the second day postoperative. The photographs accompanying their paper indicate that the tubules were slightly enlarged and that more spermatocytes were present. In previous experiments reported from this laboratory (1942 a, b) pure interstitial cell stimulating hormone from sheep pituitary maintained the testis weight and the formation of spermatozoa in hypophysectomized mature rats. Work with the immature male rat was not reported at that time, nor was the great sensitivity of the hypophysecto-

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mized male rat to ICSH stressed. Leathem (1943, 1944) has studied the reaction of the rat, hypophysectomized when immature (30–36 days) to pregnant mare serum gonadotropin, when injected for a 5 day period after a 5 day postoperative period of regression. He found maintenance of the testis, as regards both weight and differentiation.

METHODS

Male rats 40 to 43 days of age, averaging 138 gm. in weight, were hypophysectomized. Injections of gonadotropic substances were begun the day of

TABLE 1. WEIGHT OF THE REPRODUCTIVE ORGANS OF NORMAL AND HYPOPHYSECTOMIZED MALE RATS TO SERVE AS CONTROL DATA

Type of rat	Body weight		Reproductive System						
			Testes		Seminal Vesicles		Prostate		Maximum degree of testicular differentiation
	No. of rats		No. of rats	Weight	No. of rats	Weight	No. of rats	Weight	
Normal 40–43 days old	493	gm 138±1.02*	246	mg 1345±16.3	247	mg 42±0.98	247	mg 129±1.82	Spermatids
Hypophysectomized 55–58 days old 15 days P.O.	242	123±0.91	242	453± 8.5	220	18±0.32	220	48±0.65	Spermatocytes
Normal 55–56 days old	54	215±2.44	52	2355±45.6	52	255±15.9	54	305±12.5	Spermatozoa in testis and epididymis

* Standard Error of the mean.

hypophysectomy and were continued for a 15 day period (13 injections). The experiments were therefore designed to test maintenance rather than repair of the reproductive system. The testis of the rat at the time of operation had differentiated to the point where spermatids with dark staining, elongated heads were present. The interstitial cells were rounded but not distinctly epithelioid. Fifteen days after operation the testes of the hypophysectomized controls had regressed markedly. The tubules were small; the most highly differentiated cells present were a few spermatocytes; these desquamated without further differentiation. The interstitial tissue was markedly atrophic with pyknotic nuclei. At the end of the experimental period (at 55 days of age) the testis and epididymis of normal controls contained spermatozoa. Table 1 shows the weights of the reproductive organs at

TABLE 2. MAINTENANCE OF THE REPRODUCTIVE SYSTEM OF HYPOPHYSECTOMIZED 40 DAY OLD MALE RATS BY PREGNANT MARE SERUM GONADOTROPIN

Daily dose for 15 days IP		No. of rats	Reproductive system						
			Testes		Seminal vesicles		Prostate		Epididymis
μg	RU		mg	Range	mg	Range	mg	Range	
50	7.1	5	2445	2215–2840	1262	1180–1330	575	474–753	Spermatozoa
35	5	5	2523	2378–2612	1187	992–1400	542	472–617	Same
7	1	4	2080	1646–2080	206	39–472	245	115–422	Same
5	0.71	5	1712	1280–2340	97	31–180	141	77–194	Same
2	0.29	5	1572	1222–1825	27	20–36	76	44–100	Same
0.5	0.071	4	539	362–690	16	11–22	37	27–52	No spermatozoa
0	0	242	453	184–832	18	8–31	48	18–89	No spermatozoa

TABLE 3. MAINTENANCE OF THE REPRODUCTIVE SYSTEM OF HYPOPHYSECTOMIZED 40 DAY MALE RATS BY HUMAN CHORIONIC GONADOTROPIN

Daily dose for 15 days			No. of rats	Reproductive system							
				Testes		Seminal vesicles		Prostate		Epididymis
μg	RU	Route		mg	Range	mg	Range	mg	Range	
500	10	Subc.	5	1710	1452–1890	998	747–1247	586	434–790	Spermatozoa in all
		IP	5	1673	1405–2143	399	142–646	362	248–499	Same
100	2	Subc.	5	1800	1444–2166	233	61–497	229	109–360	Same
		IP	5	1638	1354–2120	127	53–346	163	105–267	Same
50	1	Subc.	5	1716	1374–2136	217	73–424	211	139–360	Same
		IP	3	1536	1284–1708	202	52–338	221	98–362	Same
25	0.5	Subc.	5	1532	1158–2076	75	38–124	137	91–192	Same
		IP	4	1594	888–2224	49	21–73	109	73–178	Same
10	0.2	Subc.	5	1076	600–1574	21	11–26	54	39–69	Same
		IP	5	1308	1124–1538	23	20–29	78	65–86	Same
5	0.1	Subc.	4	1102	484–1784	20	14–26	47	32–67	Spermatozoa in 2 of 4 rats
		IP	5	1062	808–1325	20	17–22	54	45–60	Spermatozoa in 4 of 5 rats
2	0.04	Subc.	5	414	222–676	15	12–18	43	39–52	No spermatozoa
		IP	5	536	452–600	15	13–17	40	30–47	No spermatozoa
0	0		242	453	184–832	18	8–31	48	18–89	No spermatozoa

onset of the experimental period (time of operation), the condition 15 days postoperative, and the amount of development which would normally ensue in the 15 day period, by 55 days of age. The body weight of operated rats is shown for the critical periods i.e. at age of hypophysectomy and 15 days postoperative. The body weight loss of hypophysectomized rats averaged about 18 gm. in the 15 day period. The increase in body weight of the normal rat during this period is also shown. As body weights of injected groups of rats were not influenced by the hormone preparations administered and did not deviate from the standards given in Table 1, body weights are not recorded in Tables 2 to 4.

TABLE 4. MAINTENANCE OF THE REPRODUCTIVE SYSTEM OF HYPOPHYSECTOMIZED 40 DAY OLD MALE RATS BY PITUITARY INTERSTITIAL CELL STIMULATING HORMONE

Daily dose for 15 days IP		No. of rats	Reproductive system						
			Testes		Seminal vesicles		Prostate		Epididymis
μg	RU		mg	Range	mg	Range	mg	Range	
1000	100	5	1118	852–1586	74	37–142	193	113–353	Spermatozoa in 3 of 5
250	25	5	1031	666 –1440	56	32–82	139	99–167	Spermatozoa in 2 of 5
50	5	5	1472	1200–1760	65	25–167	151	98–228	Spermatozoa in all
10	1	8	1065	442–1738	32	15–61	92	49–204	Spermatozoa in 6 of 8
2.5	0.25	5	743	724–908	19	15–25	59	45–74	Spermatozoa in 1 of 5*
0.0	0	242	453	184–832	18	8–31	48	18–89	None

* The single rat of the group in which spermatozoa were found in the epididymis had seminal vesicles weighing 22 mg., a prostate weighing 74 mg. These figures are, to be sure, near or at the maximum for the group but are within control range and were considered evidence for only an insignificant amount of male sex hormone.

PREGNANT MARE SERUM GONADOTROPIN

The pregnant mare serum hormone was administered intraperitoneally.[2] The minimum effective dose of the preparation used[3] was

[2] Leathem (1943, 1944) found that intraperitoneal injection of this hormone gave greater stimulation of the accessory organs of the male than subcutaneous injection when given at high dose levels but not at minimal doses.

7 μg., as judged by follicular development and estrous uterus in normal immature female rats, when divided in three daily intraperitoneal doses followed by autopsy in 72 hours. Table 2 shows that it was necessary to give extremely low doses to the hypophysectomized male rat in order to determine the minimum testicular response. It will be seen that at 0.5 μg. or 0.07 RU, the reproductive organs regressed in weight to the level of the hypophysectomized controls. Microscopic examination of the testes, however, showed even here some maintenance of the testicular tubules. They appeared healthier than in the hypophysectomized controls and still contained a few spermatids; the interstitial cells were not quite so atrophic as in controls. At a 2 μg. dose (0.29 RU) the testis was maintained at the operative weight or increased in weight. Differentiation had continued and spermatozoa were present in the epididymis just as in normal rats of that age. In contrast to this, there was scarcely any development in the accessory sex organs. They were only slightly heavier than those of hypophysectomized controls. At 5 μg. (0.7 RU) daily dosage, the effect on the accessories was more pronounced, and the testis weight was definitely greater than that of the 41 day male rat. A dose of 7 μg. (1 RU) was necessary to cause development of the testis and accessories comparable to that of the normal 55 day old rat. At doses of 35 to 50 μg. (5 to 7 RU) hypertrophy of the accessories was observed.

HUMAN CHORIONIC GONADOTROPIN

The preparation of human chorionic gonadotropin used[3] was not so potent per milligram as the pregnant mare serum gonadotropin, but by reference to the unitage injected, satisfactory comparisons of the effects may be made. The dose of the urinary preparation (RU) which, when given daily subcutaneously for 4 days stimulated large follicles and estrous uterus in normal immature rats, was 50 μg. Comparisons have been made here between the results of subcutaneous and intraperitoneal injections in the male rat.[4] As will be seen in Table 3 there were no marked differences in the response of the reproductive system of the hypophysectomized male rat attributable to differences in route of injection.

At 2 μg. daily doses (0.04 RU) the testicular tubules were like those of hypophysectomized controls, except in one rat out of five, in which some tubules were far more advanced than others, even containing well differentiated spermatids. At 10 or 25 μg. doses (0.2 to 0.5 RU) there was very considerable differentiation of the testis. The occurrence or extent of development was more erratic (i.e. occurred in

[3] We wish to thank the Cutter Laboratories for the pregnant mare serum gonadotropin used (Gonadin); also the Winthrop Chemical Company Inc. for the preparation of human chorionic gonadotropin (Prolan).

[4] Evans, Simpson and Jensen (unpublished) found that in immature female rats, human chorionic gonadotropin was potent at the same minimum dose whether given subcutaneously or intraperitoneally.

fewer animals) at 0.2 RU than at 0.5 RU. The testes of some animals receiving the 0.5 RU dose were considered within the normal range of development for 53-55 day old males (but did not have the maximum size of tubule development which may be seen in the normal rat of this age). At 50 and 100 μg. levels (1–2 RU) the testicular tubules of practically all rats were within normal range of development for 53–55 day rats and some showed maximum development for this age (large uniform tubules, large tubular lumens, and coarse whorls of spermatozoa).

The interstitial cells seen in rats receiving the 2 μg. level (0.04 RU) were atrophic in 3 of 5 rats. In the others of this group, and in groups receiving 10 μg. (0.2 RU), interstitial cells were like connective tissue cells, and in groups receiving 25, 50 and 100 μg. (0.5 to 2 RU) the Leydig cells were normal for the 55 day old rat i.e. epithelioid. At no level injected was there evidence of hypertrophy of the interstitial cells.

It should be noted that differentiation of the testes and production of spermatozoa were observed on the injection of low levels (even at 2 μg. or 0.04 RU), which had practically no effect on the development of the epididymis. This is in agreement with the observation (see Table 3) that definite increase in testicular weight occurred at doses lower than those necessary to maintain or stimulate the seminal vesicles and prostate.

PITUITARY INTERSTITIAL CELL STIMULATING HORMONE (ICSH)

The sensitivity of the testis of this test animal to pituitary interstitial cell stimulating hormone is shown in Table 4. As in the other experiments, injections were begun the day of operation and were given intraperitoneally daily for the 15 day period. The ICSH preparation used had been determined to be pure by physicochemical methods (electrophoretic and solubility studies) as well as by biological tests. As will be seen in Table 4, 10 to 50 μg. (or 1 to 5 RU)[5] was adequate to give complete maintenance of testicular weights. Differentiation of the testes had proceeded beyond the condition at operation, spermatozoa being present in the epididymis in most rats. A daily dose of only one fourth unit (2.5 μg) gave partial maintenance of testicular weight. Tubules were larger than in the hypophysectomized control, though somewhat smaller than those of the smallest normal onset control. The tubules were lined with spermatocytes and a few spermatids. In one of the five rats in the group, differentiation had proceeded to the production of spermatozoa. The nuclei of interstitial cells were pyknotic, but the cells had more cytoplasm than those of hypophysectomized controls. A dose of 10 μg. (1 RU) partially main-

[5] The minimum effective dose for repair of interstitial cells was 10 μg. in hypophysectomized 26–28 day female rats, 6–8 days postoperative, when the total dose was administered over a 4 day period in daily intraperitoneal injections.

tained the interstitial cells, and at 50 μg. (5 RU) interstitial cells were like those of the normal 55 day rat.

The lowest dose tested, 2.5 μg., which stimulated the testis, judged both by weight maintenance and tubular differentiation, did not maintain the accessory organs. A dose between 10 and 50 μg. (1 to 5 RU) gave maintenance of the weights of the prostates and seminal vesicles. A dose even of 100 RU did not give marked continuance of growth of these accessories, quite in contrast to the hypertrophy observed at the higher unitages of pregnant mare serum and human chorionic tonadotropin.

DISCUSSION

It must be noted that, although ICSH acted as a marked stimulus to testicular tubule differentiation even at a fraction of a unit daily dose, desquamation of germinal elements in excess of that seen in normal 55 day old rats occurred at low levels and a higher levels as

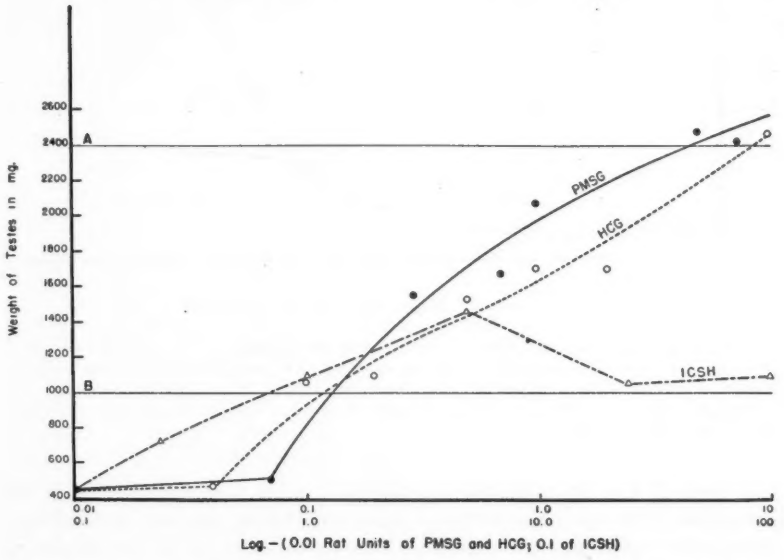

FIG. 1. Weights of testes of rats hypophysectomized at 40 days of age, after injection for 15 day periods with increasing dose levels of pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (HCG), or pituitary interstitial cell stimulaing hormone (ICSH). A: Line drawn at level of weight of testes of normal 55 day old rat. B: Line drawn at level of weight of testes at which spermatozoa are present in the testes of treated hypophysectomized rats.

well. This was true even when abundant spermatozoa were present in the testis and epididymis. The normal succession of layers of healthy differentiating cells could be seen in tubules in which desquamated

elements were abundant in the lumen of the tubule. The tubues werel therefore never completely normal at any dose level tested. A similar finding had previously been reported (Simpson, Li and Evans, 1942a), on injection of 2 mg. daily doses of pure ICSH into males hypophysectomized after maturity. The weight of the testis at the time of hypophysectomy (77 days) was maintained for 15 days, and innumerable motile spermatozoa were present in the epididymis at the end of

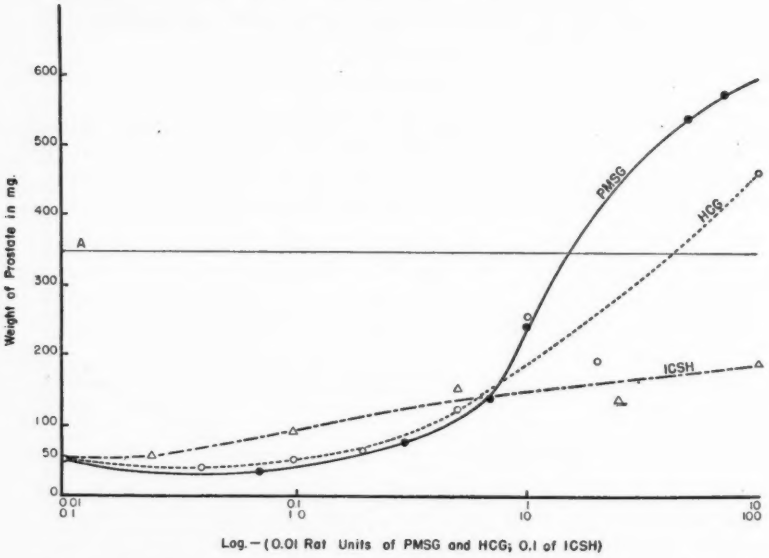

FIG. 2. Weights of prostates of rats hypophysectomized at 40 days of age, after injection for 15 day periods with increasing dose levels of pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (HCG) or pituitary interstitial cell stimulating hormone (ICSH). A: Line drawn at level of weight of prostate of normal 55 day old rat.

this period, but microscopically the testicular tubules showed some irregularity in size, the average diameter being decreased, and desquamation of immature cells was seen to be present in the midst of the formation of abundant spermatozoa.

Figures 1 to 3 compare the effect of the three gonadotropic hormones on weight of the testes, and the accessory organs (prostate and seminal vesicles). It will be noted in the chart showing testis weights that approximately 1000 mg. is indicated as the weight at which spermatozoa will be found in testis and epididymis of treated hypophysectomized males. Both curves and tables record only average weights. It will be noted in the tables that spermatozoa were found in

the epididymis at dose levels where the average testis weights did not attain 1000 mg. In these cases only the larger testes in the group, those weighing 1000 mg. or over, contained spermatozoa.

It will be remembered that in the case of all three hormones, the testes showed maintenance and differentiation at dose levels which

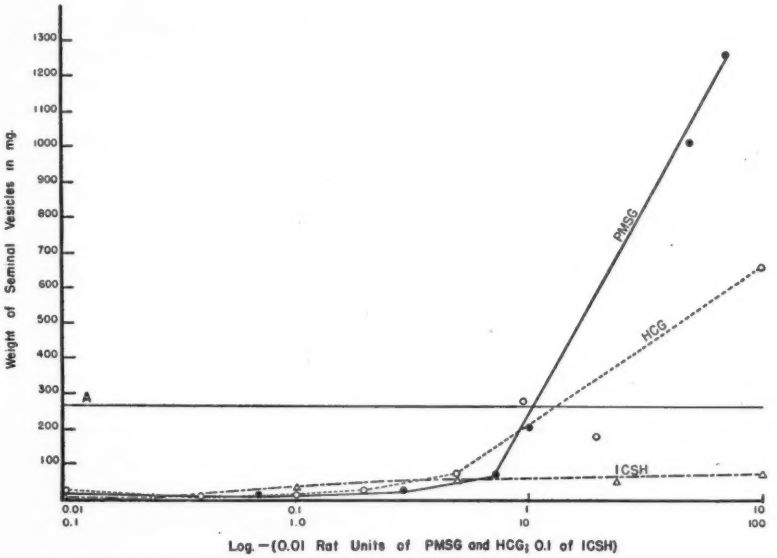

FIG. 3. Weights of seminal vesicles of rats hypophysectomized at 40 days of age, after injection for 15 day periods with increasing dose levels of pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (HCG), or pituitary interstitial cell stimulating hormone (ICSH). A: Line drawn at level of weight of seminal vesicles of normal 55 day old rat.

did not affect, or barely influenced, the interstitial cell morphology and accessory organ weights. Two of the hormones tested, human chorionic gonadotropin and pituitary interstitial cell stimulating hormone, have been considered to affect only interstitial cells and not to be gametotropic in the absence of the pituitary. When it was shown that testosterone itself was able to maintain the testicular tubules of hypophysectomized rats, the most obvious explanation for the maintenance effects of pregnancy urine hormone and pituitary interstitial cell stimulating hormone on the testes of hypophysectomized rats was that they acted through the male sex hormone secreted by the stimulated interstitial cells. The results reported here, i.e. maintenance of the testes at doses too low to effect the Leydig tissue appreciablv. is difficult to explain on the hypothesis just stated.

CONCLUSIONS

1. The testis weights of rats hypophysectomized at 40 days of age, were maintained for 15 days by gonadotropic agents (pregnant mare serum, human chorionic gonadotropin and pituitary interstitial cell stimulating hormone) given in doses less than the unit defined in the female rat (RU).

2. Differentiation leading to the formation of spermatozoa within 15 days (that is, by 55 days of age), which is approximately normal for the rat of this colony, occurred with all three hormones at daily doses approximating one fourth of a rat unit.

3. The interstitial tissue regressed when under the influence of those doses of gonadotropins which were adequate to influence the size and activity of the testicular tubules. At least one unit was necessary to maintain the interstitial cells.

4. The male accessory organs (seminal vesicles and prostate), also required higher doses for maintenance and growth than did the testicular tubules.

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WATER INTOXICATION IN RELATION TO THYROID AND ADRENAL FUNCTION[1]

ROBERT GAUNT, MARGARET CORDSEN AND MILDRED LILING

From the Department of Biology, Washington Square College of Arts and Science, New York University

NEW YORK CITY

THYROXIN IS a diuretic agent (Epstein, 1926; Marine, 1935; and Brull, 1940), particularly in diabetes insipidus (Fisher, Ingram and Ranson, 1938). It also causes other changes in renal function (Heinbecker, Rolf and White, 1943; and Eiler, Althausen and Stockholm, 1944) together with a renal hypertrophy (Hoskins, 1916).

In hyperthyroidism there is also a well known adrenal hypertrophy (Tepperman and Engel, 1942) and presumably increased adrenal cortical hormone secretion. The present experiments were originally undertaken to study the effects on water diuresis and water intoxication of agents such as thyroxin known to alter adrenal cortical function. It has been shown that variations in the body level of cortical hormones affect markedly the response of animals (Gaunt, 1944a) and man (Robinson, Power, and Kepler, 1941) to water.

The effects of hyper- and hypothyroidism on the response to excess water, and the relation of the adrenal cortex to that response, is reported here. Preliminary note has been made of part of the results (Gaunt, 1944 a, b).

METHODS

The techniques and methods of these experiments were all the same as those recently used in adrenal studies (Gaunt, 1944c). Animals fasted for 18 hours were given either 5 doses of distilled water at hour intervals, or 13 doses at half hour intervals. The resulting diuresis and symptoms of water intoxication were observed. The water dosage was calculated on the basis of body surface (3 cc. per 100 sq. cm.) and for a 200 gram rat, the size usually used, each dose was 9 cc. The water, warmed to body temperature, was given by stomach tube. Normal and treated rats were always tested simultaneously and generally in equal numbers.

Hyperthyroidism was produced by the injection of 0.2 mg. of thyroxin daily for 3 to 6 weeks. Such treatment occasionally caused death, but most animals could withstand it for many weeks. The doses used increased appe-

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tite, in some cases caused weight loss, in some cessation of weight gain, and in all other cases a reduction of the growth rate. Hyperthermia was produced, as was an obvious cardiac hypertrophy, and an adrenal hypertrophy of 53 per cent. Blood pressure was changed little if any. The relative gut weight (stomach and intestine), used in calculating water absorption, increased as a result of thyroxin treatment from 6 to 9.2 gm. per 100 gm. body weight.

An extra yeast supplement was given in the diet because of the known need for it in hyperthyroidism (Drill, 1943).

Hypothyroid rats were kindly furnished by our colleagues Drs. Gordon, Goldsmith and Charipper. The methods of MacKenzie and MacKenzie (1943), and of Astwood, Sullivan, Bissell, and Tyslowitz (1943) were used to produce a hypothyroid state. This consisted of feeding a diet containing either 0.5% thiourea or 0.2% thiouracil for a period of from 3 to 8 weeks. On this treatment the particular animals used had thyroid weights averaging 63 mg., and similar animals have been found to have an oxygen consumption depressed by 25 to 35 per cent (Gordon, Goldsmith and Charipper—unpublished).

RESULTS

Water excretion and symptoms of water intoxication in hyperthyroid rats. With any dose of water used, the rate of diuresis was strikingly

TABLE 1. WATER EXCRETION AND BODY TEMPERATURE CHANGES RESULTING FROM WATER ADMINISTRATION IN HYPER- AND HYPOTHYROID RATS

(Average Water dose = 9 cc.)

Treatment	No. animals	Per cent water excreted at following hours:							Body temperature (F°) at following hours:			
		1st	2nd	3rd	4th	5th	6th	7th	0	3rd	5th	6th
Received 5 doses of water at 0–4th hours.												
Hyperthyroid	15	50	70	76	85	86	87	91				
Hypothyroid	7	26	44	57	61	67	80	86				
Normal Controls	15	11	36	50	64	64	79	85	99.5	98.5	98.2	
Hyperthyroid-Adrenalectomized	11	18	32	34	37	34	35		100.1	98.9	97.4	
Adrenalectomized untreated*	5	14	32	35	33	30	32		98	95.9	89.1	
Received 11–13 doses of water at half-hour intervals.												
Hyperthyroid	23	26	45	50	59	61	65	73	100.1	98.4	96.3	95
Hypothyroid	7	18	24	24	30	32	41	43	97	93	85	84
Normal	20	11	23	31	35	37	41	36	99.5	93.3	88	87

* Larger series quoted by Gaunt (1944).

greater in hyperthyroid than in normal animals (Table 1), and this was associated, when large doses of water were given, with a marked protection against water intoxication. Of 28 hyperthyroid animals receiving 13 doses of water, 4 apparently died of water intoxication. Two others died after only 2 to 5 doses from causes obviously other than water intoxication—perhaps cardiac failure.

Almost twice as much water had been eliminated in hyperthyroid

animals receiving these large doses at the end of the 7 hour experiments as by normal ones. Their body temperatures, higher to start with, were better maintained than in normal animals. Most apparent, however, was the fact that most hyperthyroid animals were almost symptom free, and only rarely showed the characteristically violent convulsions of water intoxication.

Such doses of water kill about 40% of normal rats and throw nearly all cases into the full fledged syndrome of water intoxication.

There was a suggestion that hyperthyroid female rats were more resistant than males, but not enough of the former were tested to establish the point with certainty.

Water excretion and symptoms of water intoxication in hypothyroid rats (Table 1). In view of the protection afforded against excess water in hyperthyroid rats, it might be expected that the opposite effects would occur in hypothyroidism. This was not the case.

Hypothyroid animals responded in all essential respects as did normal rats to either 5 doses of water, which is non-toxic, or to large intoxicating doses. Body temperature, somewhat lower than normal initially, decreased by an amount proportional to that in normal rats. Death resulted in 6 of 7 hypothyroid cases receiving the large doses of water. This is a somewhat higher mortality than is customary in normal rats, but is identical with that of the particular normal controls run simultaneously.

Changes in blood and urine (Table 2). In normal rats large amounts of chloride are lost through the urine and by diarrhea in water intoxication and the lethal symptoms can be largely associated with that fact (Gaunt, 1944c). Despite the enormous diuresis in hyperthyroid animals the quantity of chloride lost in the urine was, both in terms of concentration and total amounts, much less than in normal rats. This difference was observed consistently at all stages and under all conditions measured, and after 13 doses of water the chloride loss was 44 per cent less in hyperthyroid than in normal animals.

This resulted in higher plasma level of chloride in the treated animals. Otherwise, the blood changes in hyperthyroid cases approximated those of normal animals, although in all instances lesser in magnitude.

The alterations in the blood included an hematocrit rise, presumably due in part to cell swelling, which together with other changes has previously been reported and discussed (Gaunt, 1944c).

Effect of adrenalectomy on the response of hyperthyroid rats to water (Table 1). After adrenalectomy the ability of hyperthyroid rats to show a hyper- or even a normal diuretic response to water was lost, and the same susceptibility to the intoxicating effects of relatively small doses of water was seen as in untreated adrenalectomized rats.

It is commonly known that hyperthyroid rats do not withstand adrenalectomy well, but in the animals used here the adrenals had

been removed only 18 hours, and the rats appeared in excellent condition at the beginning of the tests.

Hyperthyroid-adrenalectomized rats excreted water little if any faster than untreated adrenalectomized rats. They did, however, absorb water from the intestine more rapidly than ordinary adrenalectomized rats, although less rapidly than normal animals. This was shown in seven cases, where hyperthyroid-adrenalectomized rats had

TABLE 2. MEAN BLOOD CHANGES AND CHLORIDE EXCRETION IN HYPERTHYROID RATS RECEIVING WATER

Type of animal	Hematocrit	Hemoglobin	Plasma protein	Plasma chloride	Urine chloride (as Mg. NaCl)		No. animals
	%	gm.%	gm.%	M.eq.-L	3rd hr.	7th hr.	
Received 5 Doses of Water at Hour Intervals							
Hyperthyroid						12.0	15
Normal Controls						19.6	12
Samples taken after 10–13 Doses of Water at ½ Hour Intervals							
Hyperthyroid	48.2	14.6	5.17	87.8			7
Hyperthyroid					18.9	43.2	12
Normal Controls	50.3	16.2	5.16	71.0			8
Normal Controls					24.8	77.0	12
Samples taken after 5 Doses of Water at Hour Intervals							
Hyperthyroid-Adx.	57.9	17.2	4.80	85.2	6.9		7
Adx.	52.0	17.0	4.59	86.0	5.8		6
Control Fasted Animals Receiving No Water*							
Hyperthyroid	44.7 (10)	14.0 (4)	5.52 (14)	105.5 (14)			
Normal†	44.7 (23)	14.8 (30)	6.03 (13)	108.4 (21)			

* Number of cases indicated in parenthesis.
† Taken from Gaunt (1944c).

an average of 3 cc. of water per 100 gm. body weight remaining unabsorbed in the gut one hour after the fifth of 5 doses of water. At such times untreated adrenalectomized rats have about 8 cc. per 100 gm. left in the gut and normal animals have practically none (Cf. Gaunt, 1944c). It would seem, therefore, that while thyroxin partially prevented the characteristic deficiency in absorption of water after adrenalectomy, it did not at all repair the inability to excrete water which was absorbed.

Body temperature in the adrenalectomized rats which had received thyroxin was higher initially and was relatively well maintained in water intoxication, contrary to the usual condition.

Adrenal cholesterol in water intoxication. In connection with this

and related studies we have made estimations of the cholesterol content of the adrenal tissue in animals subjected to various preliminary treatments and then given intoxicating doses of water. The determinations were made by the histochemical method of Schultz (Lee, 1937).

It is generally recognized that changes in the cholesterol content of the gland are associated with the imposition of stress (Knouff, Brown, and Schneider, 1941), and this apparently reflects stimulation of the adrenal by the adrenotrophic hormone of the pituitary (Sayers, Sayers, White, and Long, 1943).

In normal animals the administration of toxic doses of water generally caused a detectable depletion of adrenal cholesterol (6 of 10 cases). Even in fatal water intoxication, however, a complete disappearance of cholesterol and hence negative Schultz tests were seen only rarely (1 of 10 cases). This was perhaps due to the fact that the experiments were acute and not of more than 7 hours duration, since in other stresses continued for longer periods we have found cholesterol characteristically absent at 24 hours but not at 6 hours (unpublished).

The adrenals of hyperthyroid animals showed either normal or perhaps in some cases slightly depleted amounts of cholesterol in their cortices. When large doses of water were given, even in the absence of intoxication symptoms, the cholesterol stores were depleted to about the same extent as in normal animals.

DISCUSSION

The experiments reported here afford another instance of the profound effect that thyroid hormone can exert on water metabolism and the results are consistent with previous knowledge (Pick, 1929–30). The ability of hyperthyroid animals to maintain a rapid and sustained diuresis when administered amounts of water that ordinarily produce water intoxication is far in excess of that seen in normal animals and slightly in excess of that seen when normal animals are protected by large doses of cortical hormone (Gaunt, 1943). The basis for this protective action is in part apparent and in part conjectural.

Hyperthyroid animals lose less chloride during water diuresis than normal animals. This effect of thyroxin presumably exerts the same protective action against water intoxication as the administration of NaCl. All who have tried it following Rowntree (1926) have found prompt relief of water intoxication to follow salt therapy—a relief not immediately due to increased water elimination. The conservation of chloride seen here accords with and in a fashion confirms the finding of Radcliffe (1943) that thyroxin reduced chloride excretion while increasing water output in diabetes insipidus. This reduced excretion of chloride in hyperthyroidism is probably not due to increased adrenal cortical activity, since the protective action of cortical hormones in

water intoxication is not associated with chloride retention (Gaunt, 1943).

It is possible, however, that the increased adrenal cortical activity, undoubtedly present in hyperthyroidism, may have an additional protective effect in water intoxication like that afforded by injections of cortical hormones in normal animals—whatever the mechanism may be. Such an idea is advanced cautiously, because it is difficult to harmonize with the experiments of Lowenstein and Zwemer (1943) on the relation of the adrenal to potassium intoxication in hyperthyroidism. They found an increased resistance to potassium only in the interval immediately following withdrawal of thyroid treatment, whereas the protection reported here against water was seen during thyroid treatment. There is almost certainly increased cortical hormone production in hyperthyroidism, but this may not result in a net increase in available hormone because of increased utilization. Whether or not increased cortical hormone was available in our cases, the resistance to water intoxication seen in hyperthyroidism was entirely dependent on adrenal function; the characteristic diuretic effects of thyroxin were not manifested in the absence of the adrenals.

It is highly probable that other direct effects of thyroxin on the metabolism of the kidney and other organs and on specific renal functions (Heinbecker, et al, 1943; Eiler, et al, 1944) were also involved in the responses seen here.

The protective action of thyroxin in water intoxication was not apparent until after 2 to 3 weeks treatment; it was, in other words, dependent upon a full-fledged hyperthyroidism. It is not clear why some comparable deficiency phenomena were not seen in hypothyroid animals, but the fact of their absence was unequivocal.

SUMMARY

A study was made of the effects of hyper- and hypothyroidism on the diuretic and intoxicating effects of excess water. The relation of the thyroid to adrenal cortical function in such effects was considered. Results follow:

1. Hyperthyroid rats showed an increased diuretic response to water and a marked resistance to water intoxication. This resistance was probably due in part but not wholly to the fact that hyperthyroid animals lose less chloride than normal ones in water diuresis.

2. In hypothyroidism, produced by thiourea or thiouracil, the diuretic response to water was normal and there was no increased susceptibility to water intoxication.

3. The characteristic effects of hyperthyroidism were largely abolished by adrenalectomy.

4. Adrenal cholesterol was depleted in both normal and hyperthyroid animals given large doses of water, and the extent of the depletion was not apparently related to the severity of the symptoms.

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THE ROLE OF THE HYPOPHYSIS AND ADRENALS IN THE CONTROL OF SYSTOLIC BLOOD PRESSURE IN THE RAT[1]

JAMES H. LEATHEM AND VICTOR A. DRILL[2]
From the Department of Zoology, Rutgers University and the Section of Physiology, Biological Laboratory, Princeton University
NEW BRUNSWICK AND PRINCETON, NEW JERSEY

THERE IS considerable evidence to show that removal of the hypophysis or the adrenals is followed by a lowering of the blood pressure. A subnormal blood pressure has been recorded for the adrenalectomized rat by both direct and indirect methods (Durant, 1928; Wyman and tum Suden, 1934; Joseph, Schweizer and Gaunt, 1943) These studies, however, were concerned with the determination of blood pressure at a specific time after adrenalectomy. Our interests were to obtain repeated determinations on the same rat over a period of two to three weeks. For this purpose an indirect method for the measurement of systolic blood pressure in the unanesthetized rat was used and repeated blood pressure determinations were recorded for each adrenalectomized rat.

Desoxycorticosterone acetate has been used extensively as replacement therapy in patients with Addison's disease and in adrenalectomized animals. This steroid will not only restore blood pressure to normal in the Addisonian but may also increase the pressure to hypertensive levels (Ferrebee, Ragan, Atchley and Loeb, 1939; Soffer, Engel and Oppenheimer, 1940; Ryan and McCullagh, 1940; Wilder, 1940; McGavack, 1941; Thorne, 1941). Administration of desoxycorticosterone acetate has been observed to increase blood pressure above normal levels in adrenalectomized dogs (Swingle, Parkins and Remington, 1941; Remington, Parkins, Swingle and Drill, 1941) in normal dogs (Kulhmann, Ragan, Ferrebee, Atchley and Loeb, 1939; Swingle, Parkins and Remington, 1941; Rodbard and Freed, 1942) and in normal rats (Grollman, Harrison and Williams, 1940; Briskin,

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[1] Part of this investigation was carried out in the laboratory of Dr. W. W. Swingle and we are indebted to him for certain necessary facilities as well as for the adrenal cortical extract.

[2] Present address: Department of Pharmacology, Yale University School of Medicine.

Stokes, Reed and Mrazek, 1943). Our investigation extends these results on the rat to include the action of desoxycorticosterone acetate on the blood pressure of adrenalectomized rats.

Following the removal of the hypophysis in the rat a decrease in blood pressure is observed (Wyman and tum Suden, 1934; Leathem and Drill, 1943; Joseph, Schweizer and Gaunt, 1943). The systolic blood pressure falls below the normal level during the first ten days after hypophysectomy after which time the blood pressure becomes stabilized and maintains a subnormal level for as long as seven months (Leathem and Drill, 1943). The subnormal blood pressure of the hypophysectomized rat may be due, in part at least, to a mild adrenal cortical insufficiency. This is suggested since it is well known that a marked atrophy of the adrenal glands occurs in the rat following hypophysectomy (Smith, 1930). To investigate this supposition we have tested the effect of desoxycorticosterone acetate and adrenal cortical extract on the blood pressure of the hypophysectomized rat. We have also administered growth hormone extracted from the anterior lobe of the hypophysis to hypophysectomized rats to observe its action on blood pressure.

The data presented in this communication show that a dosage of desoxycorticosterone acetate capable of maintaining the blood pressure of the adrenalectomized rat at a nearly normal level is very little if at all effective in the hypophysectomized rat. Large doses of adrenal cortical extract, on the other hand, do effect a partial maintenance of blood pressure following hypophysectomy but fail to maintain or restore the blood pressure to normal levels.

MATERIALS AND METHODS

Seventy-seven male rats, weighing from 198 to 303 grams and ranging from 97 to 147 days in age, were used for these experiments. The rats were maintained on purina dog chow pellets. Of this group, 14 were adrenalectomized and 37 were hypophysectomized. The blood pressure determinations were made during the period immediately following the operative procedures with the exception of a group of 9 hypophysectomized animals that were studied after a post-operative interval of six months. The systolic blood pressure was determined on the unanesthetized rat using the method of Williams, Harrison and Grollman (1939). Several pressure determinations were usually made before experimental procedures were instituted.

Desoxycorticosterone acetate (percortin)[3] was dissolved in sesame oil and administered intramuscularly in daily dosages of 0.5 mg. (0.1 cc oil). Adrenal cortical extract was concentrated so that 1 cc was equivalent to either 120 gms. of fresh gland (3x concentrate) or 200 gms. (5x concentrate) and injected subcutaneously. Growth hormone (Squibb)[3] contained 10 mg. of protein per cc and was injected subcutaneously once daily.

[3] We wish to thank Drs. E. Oppenheimer and H. W. Hays of Ciba Pharmaceutical Products for the desoxycorticosterone acetate (percortin) and Dr. J. DeFrates of E. R. Squibb and Sons for the growth hormone.

In case of the hypophysectomized rats the pituitary capsules were serially sectioned, stained with Mallory's connective tissue stain and examined microscopically to ascertain completeness of the operation. Data from only completely hypophysectomized rats are presented.

RESULTS

Normal rats. The individual systolic blood pressures of eleven normal rats fluctuated between 107 and 122 mm. Hg. over a 29-day test period. The majority of the determinations fell in the range of 111 to 115 mm. Hg. Average pressures for the group are shown in Fig. 1.

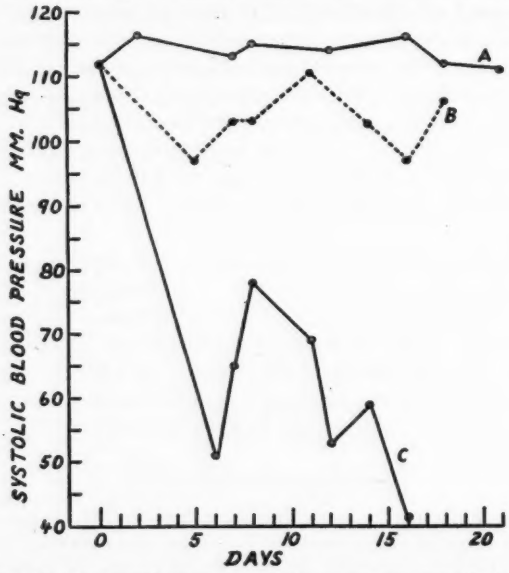

FIG. 1. Effect of desoxycorticosterone acetate on average systolic blood pressure of rats. (A) normal controls. (B) Adrenalectomized rats treated with 0.5 mg. desoxycorticosterone acetate daily. (C) Adrenalectomized controls.

Fifteen normal rats were injected with 0.5 mg. of desoxycorticosterone daily for 8 days and in this group injections were extended to 18 days in 10 rats and to 29 days in six rats. The average systolic blood pressure for the group remained within the normal range at all times, fluctuating between 111 and 118 mm. Hg. Individual cases did reveal two rats in which the blood pressure rose and remained consistently above normal. This rise was first evident after 12 injections when the blood pressure was 15 mm. above normal. The maximum pressure levels observed in each of these two animals was 140 and 153 mm.

Adrenalectomized rats. Blood pressure dropped rapidly following adrenalectomy, being 51 mm. for an average of five rats after a post-

operative interval of six days and represented an average decrease of 60 mm. The average pressure rose somewhat during the ensuing 5-day period but gradually dropped again with the death of all animals occurring 6 to 16 days after adrenalectomy (Fig. 1). The blood pressure was obtained before death in each rat and found to be below 60 mm. in every case.

Two adrenalectomized rats survived a 19-day period. Since their blood pressures suggested the presence of accessory cortical tissue these figures are not included in the above group. These rats exhibited an

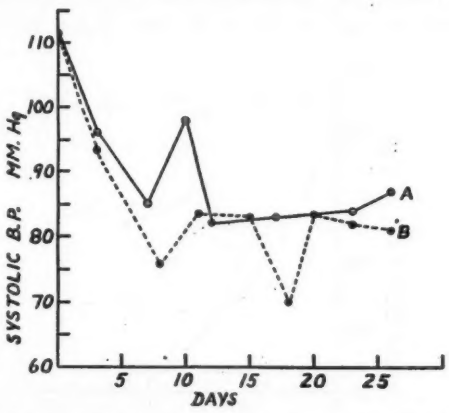

FIG. 2. Effect of desoxycorticosterone acetate on average systolic blood pressure of hypophysectomized rats. (A) Hypophysectomized rats injected with 0.5 mg. desoxycorticosterone acetate daily. (B) Hypophysectomized controls.

initial decrease in blood pressure to 60 mm. at six days after adrenalectomy. From this time, however, the pressure rose gradually to a level of 86 mm. at 15 days and at 19 days postoperatively the pressures averaged 100 mm.

Starting the day of adrenalectomy seven rats were injected with 0.5 mg. of desoxycorticosterone acetate daily for 19 days. The average systolic blood pressure for the group ranged between 97 and 111 mg. Hg. during this period as compared with the average pressure levels in normal rats of 112 to 117 mm. (Fig. 1). Blood pressure levels below 80 mm. were recorded for only one rat whereas after 7 injections of the hormone the blood pressure of two rats was found to have risen to 120–130 mm. The maximum figure recorded was 130 mm. One adrenalectomized treated rat failed to survive the entire test period.

Hypophysectomized rats. Following hypophysectomy the blood pressure dropped rapidly to an average of 76 mm. following a postoperative period of 8 days. The blood pressure became stabilized at this subnormal level which represents an average decrease of 30 to 35 mm. below the normal pressure, (Fig. 2). Individual pressure deter-

minations recorded between 10 and 30 days after hypophysectomy were found to range from 58 to 107 mm. with sixty per cent of the pressure levels falling between 73 and 87 mm.

Six rats were hypophysectomized and injected intramuscularly with 0.5 mg. of desoxycorticosterone acetate daily for 26 days starting the day of the operation. Systolic blood pressure fell to an average of 85 mm. following a postoperative interval of 7 days and appeared to be slightly above the pressure of the operated control during the following several days. The significance of these results, however, is questionable. During the ensuing period the average pressures ranged between 82 and 88 mm. or 25 to 30 mm. below normal (Fig. 2). A

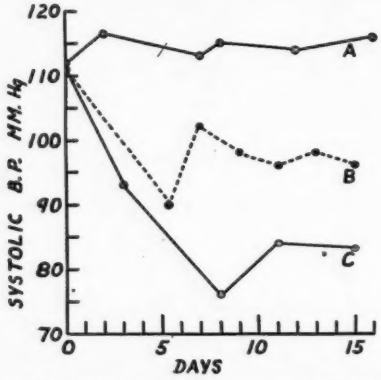

FIG. 3. Effect of adrenal cortical extract on average systolic blood pressure of rats (A) Normal controls. (B) Hypophysectomized rats injected with adrenal cortical extract. (C) Hypophysectomized controls.

partial maintenance of blood pressure was observed in one treated rat in which the normal blood pressure was 107 and 113 mm. during 12 days prior to hypophysectomy and ranged between 91 and 102 mm. during the 20 days of desoxycorticosterone acetate therapy following hypophysectomy.

To study the action of desoxycorticosterone acetate on the established subnormal blood pressure of the hypophysectomized rat three animals were injected with 2 mg. of desoxycorticosterone acetate daily for 15 days. Hormone administration was started 31 days after hypophysectomy. Prior to hormone administration the average blood pressure ranged between 78 and 84 mm. from the 11th to 31st day following hypophysectomy. When the steroid was administered the average pressure after 4 as well as after 8 days of injections was 94 mm. but on the 11th and 15th days the average pressure was 66 and 78 mm. respectively. Perhaps a slight transient rise in blood pressure resulted but the significance can be questioned. The effect was not sustained hence a return to normal pressure was not obtained.

Differences in hormone action have been noted between desoxycorticosterone acetate and an extract from the adrenal gland. Adrenal cortical extract was investigated with the possibility of obtaining more complete replacement of cortical requirements. Six hypophysectomized rats were injected subcutaneously with one cc. of adrenal cortical extract twice daily for 15 days starting the day of hypophysectomy. One cc. of this extract was equivalent to 120 gms. of fresh adrenal tissue and was designated as a 3 times concentrate. Blood pressure determinations were taken every other day from the 5th day following the removal of the hypophysis. A partial maintenance of the blood pressure was obtained as indicated by the average pressure of 102 mm. (range 97 to 110) at the 7th day and 98 mm. (range 80 to 117) at the 9th day whereas the non-treated hypophysectomized rat exhibited an average pressure of 76 mm. on the 8th (range 65 to 114) postoperative day. Furthermore the average blood pressure of these cortical hormone treated hypophysectomized rats never fell below 90 mm. during the 15-day injection period (Fig. 3). The average maintenance of pressure was approximately 15 mm. above that of the operated control. Individual determinations on the 15th postoperative day revealed only one of the six rats with a blood pressure below 90 mm. whereas 7 of 9 controls had a pressure below 90 mm. at this time.

Administration of 1 to 4 cc. of a more concentrated cortical extract (5x concentrate) per day proved no more effective in maintaining blood pressure during the first seven days after hypophysectomy than the less concentrated material. The average pressure for 3 rats was 94 mm. at this time. Injections were discontinued on the 7th day and on day 12 the pressure had decreased to 77 mm. which level compares with operated control levels and is 20 mm. lower than hypophysectomized rats treated with cortical extract.

Three rats hypophysectomized for 46 days were injected with 2 cc. of cortical extract (3x concentrate) daily for 10 days but no significant elevation of blood pressure was observed in this period.

A preparation of anterior pituitary growth hormone was also tested for a possible effect on systolic blood pressure in hypophysectomized rats. Four rats were injected subcutaneously once daily with 0.4 cc. (4 mg. protein) of growth hormone starting the day of hypophysectomy and continued for 10 days. The average systolic blood pressure had dropped to 71 mm. at day 10 and indicated the absence of effect.

Nine rats that had been hypophysectomized six months previously and whose blood pressures were stabilized at approximately 80 mm. were injected with growth hormone once daily for 31 days. The daily dosage was increased from 0.1 cc. to 0.4 cc. during the first 15 days and continued at the latter dosage for the remainder of the experimental period. The systolic blood pressure was not influenced. These

rats increased in body weight but on autopsy revealed the typically atrophic adrenal and thyroid glands indicating an absence of these pituitary factors in the extract.

DISCUSSION

It is well established that removal of the adrenal glands is followed by a fall in blood pressure. We have shown that the course of the blood pressure following adrenalectomy in the adult male rat simulates that of other animals. However, in the presence of accessory cortical tissue the blood pressure can return to normal levels.

Adrenal cortical extract and desoxycorticosterone acetate (DCA) will maintain or restore the blood pressure following adrenalectomy in experimental animals. In fact DCA has been shown to elevate the blood pressure of adrenalectomized dogs above normal levels when injected for 6 to 20 days (Swingle, Parkins and Remington, 1941; Remington, Parkins, Swingle and Drill, 1941). No correlation between blood pressure and dosage was noted. Furthermore DCA will elevate the blood pressure of normal dogs (Swingle, Parkins and Remington, 1941; Rodbard and Freed, 1942). Swingle, Parkins and Remington (1941) found the elevation of blood pressure in normal dogs with DCA to be less marked and less consistent than in adrenalectomized dogs. Rodbard and Freed (1942) obtained an increase in blood pressure in 1 of 3 normal animals. Grollman, Harrison and Williams (1940) obtained definite hypertension in normal rats by injection 0.5 mg. of DCA daily for 8 days. Similar but less marked results were reported by Briskin, Stokes, Reed and Mrazek (1943). Our experience with the 0.5 mg. daily dosage of DCA failed to reveal a hypertensive activity in normal rats injected for less than 12 days and then only 2 of 10 rats exhibited a blood pressure rise. In fact 4 rats failed to show any alteration in blood pressure over a 29-day injection period. Administration of the 0.5 mg. daily dosage of DCA to adrenalectomized rats maintained the blood pressure essentially normal with no definitely hypertensive levels recorded although 2 rats exhibited pressures of a high normal range. A hypertensive blood pressure response to DCA appeared to be the exception rather than the rule in the rat with the dosage employed.

Hypertensive levels of blood pressure are observed with DCA therapy but the cause is unknown. Grollman, Harrison and Williams (1940) suggest the hypertensive action of DCA as due to renal damage and that other systemic toxic effects may also cause a pressure rise. Swingle, Parkins and Remington (1941) cite evidence against a toxic action on the kidney as a causative factor in the hypertension induced by DCA. Our results tend to demonstrate that the action of DCA is not one of toxicity since this steroid did not alter the blood pressure of the hypophysectomized rat. On the other hand, stilbestrol which is known to be toxic in large doses readily lowers the blood pressure of

hypophysectized rats and curtails survival (Leathem & Drill, 1943). Electrolyte metabolism changes may also be responsible for the variable results. Loeb (1942) points out that salt alone can raise the blood pressure of an Addisonian above normal and Remington, Parkins, Swingle and Drill (1941) have shown that increased salt intake can augment the action of DCA on blood pressure.

Adrenal cortical extract, unlike DCA, is devoid of a hypertensive action in normal and adrenalectomized animals. This hormone too affords more complete replacement therapy in adrenal insufficiency than will DCA and as we have shown adrenal cortical extract is capable of partially preventing the fall in blood pressure following hypophysectomy in the rat. DCA, however, had little effect in preventing the fall in blood pressure in hypophysectomized rats whereas the same dose will effectively maintain blood pressure in adrenalectomized rats. While these results support our premise that the subnormal blood pressure of the hypophysectomized animal is in part due to insufficient cortical hormone they also indicate that some additional factor is responsible for the lowering of systolic blood pressure after hypophysectomy in the rat. This is further emphasized by our unsuccessful preliminary attempts to raise the subnormal hypophysectomized rat's blood pressure with adrenal cortical extract.

The role of the pituitary gland in the control of blood pressure is shown too in cases of pituitary tumor and concomitant hypertension (Cushing, 1933; Henstell, 1933). However, Brenning (1933) found the blood pressure in acromegaly to be normal. Our experiments verify the lack of effect of growth hormone on the blood pressure of the hypophysectomized rat. Blood pressure could not be maintained or restored with growth hormone.

SUMMARY

A decrease in systolic blood pressure of the rat occurred after ablation of the adrenal glands in a manner simulating that observed in other animals. Desoxycorticosterone acetate effectively maintained the blood pressure of the adrenalectomized rat and in only an exceptional case did it cause a moderate hypertension in the normal rat.

Following hypophysectomy a lowering of the blood pressure to a subnormal level was observed and the administration of desoxycorticosterone acetate failed to prevent this drop in pressure although the same dosage was able to prevent the decline in pressure following adrenalectomy. On the other hand, adrenal cortical extract did effect a partial maintenance of blood pressure in the hypophysectomized rat. The subnormal blood pressure of the hypophysectomized rat appears to be due in part to insufficient cortical hormone but these results also indicate that some additional factor is responsible for the lowering of systolic blood pressure after hypophysectomy in the rat.

Attempts to restore the subnormal blood pressure of the hypophysectomized rat to normal with adrenal cortical extract, desoxycorticosterone acetate or growth hormone were unsuccessful. Furthermore, growth hormone did not prevent the fall in blood pressure when administered immediately following hypophysectomy.

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METABOLISM OF THE STEROID HORMONES. ADRENAL CORTICAL-LIKE MATERIAL IN HUMAN URINE[1]

RALPH I. DORFMAN, BENJAMIN N. HORWITT AND REGINALD A. SHIPLEY

From the Brush Foundation and Departments of Biochemistry and Medicine, Western Reserve University School of Medicine and Lakeside Hospital

CLEVELAND, OHIO

IT HAS BEEN established from recent studies in this laboratory that an extract may be obtained from the urine of normal men which has physiological properties identical with those of adrenal cortical compounds. The extract shows the following activities when tested on the adrenalectomized rat: it protects against exposure to cold (Dorfman, Horwitt and Fish, 1942); it elevates liver glycogen (Horwitt and Dorfman, 1943); it maintains life (Dorfman and Horwitt, 1943); it prolongs muscle work performance (Shipley, Dorfman and Horwitt, 1943); and it prevents water intoxication (Schiller and Dorfman, 1943).

Evidence that urine contains adrenal cortical-like material was brought forward by the finding of Perla and Marmorsten-Gottesman (1931) that a benzene extract of human urine would increase the resistance of adrenalectomized rats to histamine. Shortly afterward, Grollman and Firor (1932–33) reported that benzene soluble material obtained from urine would prolong life when administered to adrenalectomized rats. Anderson, Haymaker and Joseph (1938) reported that a similar extract obtained from the urine of patients with Cushing's syndrome was potent when assayed for its capacity to maintain life in the adrenalectomized rat, whereas extracts of normal urine were inactive. Weil and Browne (1939a, 1939b) obtained from the urine of patients recently subjected to surgical procedures, and from other patients suffering from various diseases, an ethylene dichloride extract which protected adrenalectomized rats from the lethal effects of exposure to cold. The urine from normal adults showed no activity. Venning, Hoffman and Browne (1943) have also recently demonstrated that extracts of urine obtained from postoperative patients possess life maintaining and glycogenic properties.

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It is the purpose of this communication to present data bearing upon the urinary content of cortin-like material as encountered in normal men and women and in patients showing evidence of hypofunction of the adrenal cortex. Recovery of active material in the urine after the administration of cortical hormones has also been investigated.

METHODS AND SUBJECTS

In most instances, pooled 3 to 6 day specimens of urine were collected. Toluene was added as a preservative. Extraction was performed by a technique similar to that previously employed by Weil and Browne (9). Three extractions were made at room temperature with one part of ethylene dichloride to four parts of urine. The ethylene dichloride was evaporated in vacuo at an internal temperature below 40°C. After thrice adding absolute ethanol and evaporating to dryness to remove all traces of ethylene dichloride, the final brownish oil was dissolved in 10% ethanol. It was administered by stomach tube to male rats (Sprague-Dawley strain) weighing between 35–50 grams which had been adrenalectomized the day before. Groups of 9 to 11 animals were used for an assay. Run in parallel was always a similar control group which received 10% ethanol. Immediately after administration of extract, the rats were enclosed in individual glass jars and placed in a cold room maintained at $5.5° \pm 1°$C. Observations were made at 30 minute intervals and were continued for at least 12 hours. An occasional rat which had received active material would live longer than this, but none were followed beyond 14 hours. Rats were considered dead when they showed no movement after agitation of the jar.

In the assay procedure, activity was manifested by an increase in the average number of hours that life was prolonged in the experimental groups when compared to the average survival in the control group. Significance of the difference between mean survivals were calculated from Fischer's "t" table. The cold protection test was chosen in preference to other methods of assay because of its simplicity and sensitivity.

Subjects studied were 9 normal men, 8 normal women and 7 patients with Addison's disease. The patients with Addison's disease were studied in this hospital and details concerning five of them may be obtained from another publication (Shipley, 1944). All 7 Addisonian patients exhibited the typical findings usually encountered in the disease. These patients with Addison's disease were in good condition at the time urine was collected. They were usually maintained with desoxycorticosterone acetate pellets or salt.

RESULTS

Cortin-like material was invariably demonstrated in the urine of both normal men and women (Tables 1 and 2). In the group of patients with Addison's disease, cortin-like material was demonstrated only once in 15 assays on 7 patients (Table 3). A second sample of urine on patient H. L., whose urine had previously shown a positive response, was found to be negative. Extracts from normal urine will usually evoke significant responses when administered in doses equivalent to 4 to 6 hours of excretion. In the patients with Addison's disease, how-

TABLE 1. CORTIN-LIKE MATERIAL IN THE URINE OF NORMAL MEN
(Each group consisted of 9 to 11 Adrenalectomized Rats)

Age of subject	Urine equivalent administered per rat (hours)	Control rats Mean survival hours	Urinary extract Treated Rats Mean survival hours	Increase in mean survival %	P
24	5.4	7.1 ±0.37*	11.0 ±0.40	55	0.01
24	5.4	6.6 ±0.39	8.6 ±0.46	30	0.01
25	5.4	4.8 ±0.19	6.8 ±0.56	42	0.01
26	5.8	5.9 ±0.46	8.1 ±0.34	37	0.01
26	6.0	5.9 ±0.46	8.7 ±0.47	48	0.01
29	5.4	4.8 ±0.19	7.2 ±0.33	50	0.01
31	6.0	5.9 ±0.46	8.9 ±0.59	51	0.01
43	5.4	6.1 ±0.45	9.0 ±0.93	48	0.02
44	5.4	7.1 ±0.37	10.1 ±0.53	42	0.01

* Standard Error

ever, no significant response was observed even when equivalents of 10 to 12 hours of urine were administered to the test animals on four occasions.

When it was discovered that no cortin-like material was demonstrable in the urine of individuals with Addison's disease, it became evident that these patients would serve as excellent subjects to which adrenal cortical compounds might be administered and the urine assayed for active substances that might be excreted. Four patients were selected for these studies, (Table 4). Three patients who had received desoxycorticosterone acetate[2] orally in doses ranging from 150 to 675 mg. excreted no active material. In three out of five instances after the administration of Wilson's cortical extract, significant activity was detected in the urine.

TABLE 2. CORTIN-LIKE MATERIAL IN THE URINE OF NORMAL WOMEN
(Each Group Consisted of 10 Adrenalectomized Rats)

Age of subject	Urine equivalent administered per rat (hours)	Control rats Mean survival hours	Urinary extract treated rats Mean survival hours	Increase in mean survival %	P
21	4.1	6.0 ±0.23*	8.5 ±0.62	42	0.01
22	5.4	4.6 ±0.42	6.7 ±0.48	46	0.01
23	5.4	5.5 ±0.23	7.2 ±0.59	31	0.02
24	5.4	4.8 ±0.19	7.3 ±0.83	52	0.01
24	5.4	6.0 ±0.23	7.5 ±0.65	25	0.05
25	5.4	7.5 ±0.62	10.5 ±0.74	40	0.01
32	5.4	4.8 ±0.19	6.8 ±0.71	42	0.01
35	5.4	7.5 ±0.62	10.8 ±0.44	44	0.01

* Standard Error

[2] We wish to thank Ciba Pharmaceutical Products, Inc. and the Schering Corporation for the desoxycorticosterone acetate.

TABLE 3. CORTIN-LIKE MATERIAL IN THE URINE OF PATIENTS WITH ADDISON'S DISEASE
(Each Group consisted of 9 to 12 Adrenalectomized Rats)

Patient and sex	Urine equivalent administered per rat (hours)	Control Rats Mean survival hours	Urinary extract treated rats Mean survival hours	Increase in mean survival %	P
A.G. ♂	6.0	6.8±0.57*	7.9±0.57	+16	>0.05
	6.0	5.7±0.32	4.8±0.27	−19	—
	7.2	5.0±0.27	4.8±0.30	− 4	—
	8.3	5.0±0.27	4.8±0.18	− 4	—
R.P. ♂	6.0	5.7±0.32	5.2±0.28	− 9	—
	10.8	7.4±0.28	7.4±0.50	0	—
J.B. ♂	6.0	6.1±0.39	6.2±0.30	+ 2	—
	6.0	6.7±0.66	6.7±0.61	0	—
	12.0	4.6±0.42	5.0±0.44	+9	>0.05
G.L. ♀	12.0	6.6±0.39	6.3±0.55	− 5	—
	6.0	5.7±0.32	5.2±0.40	− 9	—
M.W. ♀	8.3	5.5±0.44	5.4±0.57	− 2	—
J.P. ♂	11.2	5.9±0.21	4.5 ±0.30	−24	—
H.L. ♂	6.0	6.8±0.57	9.9±0.47	+46	<0.01
	6.0	5.7±0.32	5.2±0.40	− 9	—

* Standard Error

DISCUSSION

In view of the fact that human urine contains an unidentified material which has the physiological properties of adrenal cortical hormones, and in light of the evidence now presented that this material is decreased or absent in the urine of individuals with adrenal insufficiency and reappears after the administration of adrenal cortical extracts, it seems reasonable to assume that it has its origin in

TABLE 4. CORTIN-LIKE MATERIAL IN THE URINE OF PATIENTS WITH ADDISON'S DISEASE AFTER THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACT AND DESOXYCORTICOSTERONE
(Each Group Consisted of 9 to 11 Adrenalectomized Rats)

Patient and sex	Adrenal cortical extract Oral (cc)	IV (cc)	IM (cc)	SC (cc)	Desoxycorticosterone acetate oral (mg)	Duration of treatment (days)	Urine equivalent administered per rat (hours)	Control rats Mean survival hours	Urinary extract treated rats Mean survival hours	Increase in mean survival %	P
A.G. ♂	126	—	—	—	—	1	2.2	5.0±0.27*	4.4±0.35	−12	—
	—	—	—	—	150	1	2.2	5.0±0.27	5.1±0.43	+ 2	>0.05
R.P. ♂	777	—	—	—	—	5	8.1	6.7±0.64	8.9±0.70	+33	<0.05
	—	—	—	—	675	6	10.8	6.7±0.64	6.6±0.57	− 2	—
J.B. ♂	—	10	10	—	—	1	1.8	6.6±0.35	7.8±0.37	+18	<0.05
	—	80	30	18	—	2	4.0	6.6±0.35	9.7±0.62	+47	<0.01
M.W. ♀	144	—	—	—	—	2	5.3	5.5±0.44	5.4±0.59	− 2	—
	—	—	—	—	300	2	4.0	4.8±0.19	5.3±0.26	+10	>0.05

* Standard Error

the adrenal cortex. A similar conclusion was arrived at from the study of cortin-like material in the urine of monkeys. Intact and gonadectomized male and female monkeys excreted cortin-like material while adrenalectomized monkeys did not (Dorfman, Horwitt, Shipley and Abbott, 1944). Furthermore, by assaying for the material, one should be able to assess cortical function more satisfactorily than is now possible by any other method. Although variations in urinary 17-ketosteroids may, to a certain degree, reflect adrenal cortical activity, the parallelism is rather crude. The 17-ketosteroids are part of a metabolic waste basket which remains to be properly sorted and the contributing sources identified. These compounds, of course, arise only in part from the adrenal glands and, in fact, there may be additional sources other than the gonads.

In addition to the direct evidence that "urinary cortin" is actually of adrenal origin, there is also good reason to suspect that hormones arising from the gonads could not be responsible for the activity. Certainly, none of the known metabolites of sex hormones would produce the observed activity.

SUMMARY

By means of a cold protection test using adrenalectomized rats, cortin-like material was demonstrated in the urine of nine normal men and eight normal women. Cortin-like material could be demonstrated only once in fifteen assays in urines from seven patients with Addison's disease.

It seems justified to assume that this material arises in the adrenal cortex, and that variations in urinary content reflect the physiological activity of the gland.

The administration of adrenal cortical extracts to patients with Addison's disease was reflected in the excretion of cortin-like material. Orally administered desoxycorticosterone had no such effect.

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NOTES AND COMMENTS

EFFECT OF THYROXINE ON THE NEUTRAL FAT AND CHOLESTEROL CONTENT OF THE BODY AND LIVER OF RATS

SINCE THYROXINE is such an important metabolic stimulant is was felt that its administration might affect the deposition of fat in the livers of animals on a low choline intake. In order to test this hypothesis young albino rats were put on a choline-free, low-protein, high-carbohydrate diet for periods varying from 15 to 42 days, after which some of them were given thyroxine, some choline and other choline plus thyroxine.

A liquid diet containing insoluble material in suspension, Diet 1, was used throughout the depletion period. Our experience has been that, as a rule, the animals on the deficient diet ate this liquid diet better than if it were fed in the dry state. The required amount of the diet was transferred daily to the feeding cups by means of a 15 cc. Folin and Wu pipet with a broken tip. The amount fed was controlled so that it was completely consumed each day and each animal in an individual experiment received the same amount per 100 grams of body weight. The amount fed in the individual experiments differed slightly from one experiment to another depending upon the general appetite of the groups. At the end of the depletion period the animals were divided into two or three groups and fed Diet 2, plus supplements of choline, thyroxine, or choline plus thyroxine for a period of 9 to 11 days. During this period each animal's food allowance was based on its weight at the beginning of the supplemental feeding rather than on its actual daily weight. At the end of the period the animals were rapidly exsanguinated by decapitation. Their livers were removed, individually weighed, and after grinding in a mortar samples were taken and analyzed for their lipid content. The analytical procedures have been described previously. Outhouse and Forbes (1940). After removing the intestinal tract and skin from each animal, the bodies from each experimental group were ground together in a meat chopper, transferred to a beaker, weighed, and dried in an oven at 100° C. The skins from each group were similarly treated except that they were not ground prior to drying. After thorough drying, the bodies were reweighed, ground thoroughly, mixed, and samples were taken for lipid analyses. The skins, after drying, were put through a meat chopper, after which they were thoroughly pounded in a mortar, mixed and analyzed. Absolutely thorough mixing of the skins is impossible, consequently the values obtained for them are considered as approximate only:

DIET I

Sucrose	1000 gm.
Casein, vitamin free	100

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Salt mixture without KI (Wesson 1932)—	30
Cellu flour B	8.5
Percomorph oil, 50%	15 drops
Thiamine HCl	2.5 or 4.5 mg.
Potassium iodide	0 or 1.8 mg.
Water	800 cc.

Diet 2 was made as above except that no water was used and vitamins were added as follows: riboflavin 8 mg., pyridoxin hydrochloride 6 mg., calcium pantothenate 15 mg., and niacin 14 mg. In order to be able to demonstrate any effect of thyroxine on the level of liver fat in the presence of choline, the choline intake unless otherwise specified, was kept sufficiently low, 25 mg. per 100 gm. of food, to allow the production of moderately fatty livers in the majority of the experimental animals receiving choline alone. Thyroxine was administered at a level of 1 mg. per 100 gm. of diet.

In experiments 4, 6 and 10 no iodine was added to the diet in the hope that differences between the control and treated animals would be increased. The response did not differ, however, from that obtained when iodine was present. It is possible that a state of iodine deficiency may not have been produced as the animals were in an air conditioned room with forced circulation, along with stock animals on Rockland rat pellets, and sufficient dust from the latter may have been carried by the circulating air to prevent a state of definite iodine deficiency. Since the thiamine requirement of rats is known to be definitely increased in hyperthyroidism, the thiamine content of the diet was increased in experiments 4, 5 and 9 to 4.5 mg. per kilogram of sucrose in the diet, but no apparent difference was noted.

From the experimental results presented in Table 1, it will be seen that the level of both neutral fat and cholesterol in the livers of the animals fed a diet containing both choline and thyroxine was distinctly lower on the whole than that of corresponding animals receiving similar amounts of choline. Experiment 3 can be excluded from consideration as in this set of animals the choline itself was sufficient to maintain a normal liver fat content and any additional effect of thyroxine could not be expected. No satisfactory explanation, however, can be offered for the discrepancy noted in experiment 4. However, in spite of this discrepancy the general tendency is quite definite.

The neutral fat content of the livers of the animals receiving thyroxine alone was always fairly high. Thus any lowering effect of thyroxine in the absence of choline on liver fat must be very slight, if any. The absence of inositol and essential fatty acids from the diet may account for the fact that in experiments 11 and 12, where choline was fed throughout the whole experimental period, the neutral fat content of the livers was, even in the absence of thyroxine, only slightly above normal while the cholesterol concentration was definitely elevated.

The concentration of neutral fat plus cholesterol in the bodies and skins of the thyroxine-choline fed animals was in all experiments less than that of the corresponding animals receiving the same amounts of choline per 100 grams of body weight. Calculated on a wet basis, the average values for the bodies were 7.1 per cent for the thyroxine-choline fed animals and 8.9 per cent for those receiving choline alone. Those receiving thyroxine alone

showed, on the whole, intermediate values. The skins invariably gave values in the same general direction as those of the corresponding bodies.

The total cholesterol content of the animals did not seem to be affected by thyroxine administration, a result in agreement with the findings of

TABLE 1. EFFECT OF CHOLINE AND THYROXINE ON NEUTRAL FAT AND CHOLESTEROL CONTENT OF LIVER

Experiment number	Number of rats	Days on choline deficient diet	Average weight*	Average daily gain in weight**	Daily food intake per 100 gm. body wt.**	Liver				Supplements
						Neutral fat		Cholesterol		
						% wet wt.	Per 100 gm. body wt.	% wet wt.	Per 100 gm. body wt.	
			gm	gm	gm		gm		gm	
1	3	23	87	1.6	12	3.5	.146	.34	.014	Choline + thyroxine
	3		79	2.9	12	16.3	.968	.96	.057	Choline
	4		80	1.2	12	18.2	.874	.86	.041	Thyroxine
2	4	20	82	2.0	15	3.5	.161	.34	.016	Choline + thyroxine
	4		76	2.6	15	5.9	.344	.54	.031	Choline
	2		83	1.8	15	13.4	.885	.69	.045	Thyroxine
3	4	28	76	1.6	14	2.4	.104	.65	.028	Choline + thyroxine
	3		86	2.9	14	2.0	.097	.55	.027	Choline
	3		89	1.6	14	13.4	.721	.90	.048	Thyroxine
4	4	22	83	2.4	15	6.1	.250	.31	.012	Choline + thyroxine
	3		84	2.9	15	4.7	.240	.29	.015	Choline
	3		88	1.5	15	19.9	1.000	.66	.033	Thyroxine
5	4	32	79	2.0	15	6.0	.287	.36	.017	Choline + thyroxine
	3		84	3.1	15	9.4	.527	.57	.032	Choline
	3		84	1.1	15	12.8	.676	.87	.046	Thyroxine
6	4	21	84	0.8	15	3.3	.129	.27	.010	Choline + thyroxine
	3		79	2.5	15	19.0	1.063	.91	.051	Choline
	2		81	1.6	15	15.2	.978	.57	.036	Thyroxine
7	4	15	70	1.3	14	5.2	.191	.64	.023	Choline + thyroxine
	3		78	2.0	14	8.1	.409	.79	.040	Choline
8	4	33	80	1.7	14	6.0	.258	.92	.039	Choline + thyroxine
	5		80	2.5	14	13.8	.741	1.46	.078	Choline
9	5	38	79	2.2	15	7.0	.313	.76	.034	Choline + thyroxine
	5		76	3.1	15	12.5	.730	1.06	.062	Choline
10	8	42	97	1.1	11	4.0	.161	.77	.031	Choline + thyroxine
	8		103	2.2	11	6.8	.322	.87	.040	Choline
11	3	43	87	0.3	10	2.5	.099	.52	.020	Choline + thyroxine
***	2		82	0.7	10	4.6	.168	1.02	.037	Choline
12	5	52	60	0.1	—	2.3	.084	.63	.023	Choline + thyroxine
****	5		60	0.6	—	3.3	.119	.74	.027	Choline

* Beginning of supplemental feeding.
** During administration of supplements.
*** 2.5 mg. of choline chloride per 100 gms. of diet was fed throughout the whole experimental period of 43 days. Thyroxine was fed for the last 10 days only.
**** Choline chloride was fed throughout the whole experimental period at a level of 100 mg. and thyroxine at a level of 1 mg. per 100 gms. of diet.

Fleischmann and Shumacker (1942). However, when thyroxine was given along with choline, at least in short term experiments, it tended to keep down the concentration of liver cholesterol as well as that of neutral fat. On the other hand, in the absence of choline thyroxine exerted little, if any, effect on either liver neutral fat or cholesterol content.

SUMMARY

The administration of thyroxine to rats on a high-carbohydrate, low-protein, fat-free, moderate-choline diet tends to keep the neutral fat and cholesterol content of the livers less than that of control animals receiving

corresponding amounts of choline. In the absence of choline the neutral fat content of the livers of the thyroxine fed animals was invariably quite high indicating that choline must be present in order for thyroxine to exert definite lipotropic action.

J. C. FORBES

From the Department of Biochemistry
Medical College of Virginia,
Richmond, Virginia.

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SOLUBILITY OF CERTAIN STEROIDS AND OTHER WATER-INSOLUBLE SUBSTANCES IN AQUEOUS SOLUTIONS OF SODIUM DEHYDROCHOLATE[1]

ESTRONE, estriol and alpha-estradiol are regarded as being practically insoluble in water. The only accurate information on this point indicates that the maximum solubility of estrone is 2.1 mg. per liter of water (Butenandt and Westphal, 1934). Studies reported previously from this laboratory indicate that relatively large amounts of exogenous and endogenous estrogen can be excreted in the bile of dogs and human subjects (Cantarow et al., 1942, 1943). Inasmuch as almost all of this is in the free state, the manner in which it is kept in solution became a matter of interest. Attention was naturally centered first upon the possible influence of the bile acids, because of their well-known effect in enhancing the solubility of many poorly soluble substances. We are presenting here the results of preliminary studies of the influence of sodium dehydrocholate in this connection. Results obtained with estrogens led us to extend our observations to include other steroids and certain non-steroid compounds.

Results and Comment. Data regarding the solubility of several substances investigated in aqueous solutions of sodium dehydrocholate are presented in Table 1. In all instances, vigorous boiling from ½–2 minutes was necessary to effect complete solution of the quantities of substance indicated in the table, although a considerable portion went into solution immediately upon boiling. Naphthalene was found to be freely soluble in 20% solution of sodium dehydrocholate and 2-methyl-1, 4-naphthoquinone in 5% solution; cholesterol and estradiol benzoate were only very slightly soluble.

These solutions almost invariably remained clear after cooling. In some instances, but not consistently, a precipitate appeared after cooling and standing at room temperature for several hours. This precipitate did not resemble the original dissolved substance, being lighter and more bulky and

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flaky. Re-solution occurred on boiling. A similar precipitate frequently appeared upon diluting the original solution with either distilled water or phosphate buffer at pH 8. This would often not re-dissolve upon boiling, but did so invariably after addition of varying quantities of 20% dehydrocholate solution and boiling.

Bioassay revealed that the estrogenic activity of the estrone and alpha-estradiol solutions and the androgenic activity of the testosterone solution were at least equal to that of equal quantities of these hormones in oil. Assay

TABLE 1. SOLUBILITY IN AQUEOUS SOLUTIONS OF SODIUM DEHYDROCHOLATE (pH 8.5)

Substance* 1 mg.	Volumes of sodium dehydrocholate solution					
	20%	10%	8%	6%	4%	2%
	cc.	cc.	cc.	cc.	cc.	cc.
Alpha-estradiol	1	1.2	1.2	1.3	>2	>20
Estrone	1	1.3	1.3	1.5	>4	
Estriol	1					
Progesterone	1	1.4			>6	
Androsterone	1	1.3				
Testosterone	1	1.2				
Diethylstilbestrol	1					
Methylsbilbestrol	1					
Desoxycorticosterone acetate	1.3	1.5	1.6	2.0	>10	>20
Calciferol	5					

* We are greatly indebted to the following for generous supplies of the substances used in the present investigation: Dr. Schwenk of the Schering Corporation for estradiol, testosterone, progesterone and DCA; Mr. Mautner of the Ciba Corporation for androsterone; Mr. J. Lucas of the Winthrop Chemical Co. for the calciferol; Mr. Paul de Haen of the Riedel- de Haen Co. for the sodium dehydrocholate and sodium deoxycholate.

of the calciferol solution revealed about 60% of the expected vitamin D activity. An adrenalectomized dog has been maintained for 1 month in good condition by intravenous and intramuscular injections of 0.5 mg. of DCA in sodium dehydrocholate solution daily, the same quantity of DCA in oil having been necessary for maintenance previously.

The nature of this phenomenon is not clear. Dehydrocholic acid is not believed to form coördination compounds (choleic acids). That this principle is probably not involved is suggested by the following observations: (1) with the exception of naphthalene and vitamin K, the lowest dehydrocholute:solute ratio observed in the solutions obtained was 78:1, which greatly exceeds the highest coördination number of any of the known choleic acids (Sobotka, 1937); (2) the substances indicated in the table were practically insoluble in comparatively large volumes of 10% solutions of sodium deoxycholate under identical conditions; (3) after solution had been effected, precipitation occurred at times after addition of water or phosphate buffer solution.

The capacity of bile acids for keeping many water-insoluble substances in solution is well known. However, the following observations are difficult to explain on the basis of the operation of purely physical forces in the production of these solutions by dehydrocholate: (1) no such striking effect is produced by deoxycholate; (2) addition of lecithin, which frequently enhances this effect of bile acids, exerts no appreciable influence upon the dehydro-

cholate solutions; (3) the relative insolubility of cholesterol and estradiol benzoate in this medium as compared to the substances indicated in the table.

Estrone-20% dehydrocholate solutions containing 1 mg. of estrone per 1.2 cc. were dialyzed for 72 hours at room temperature through cellophane against (a) 3½ volumes of 20% sodium dehydrocholate solution, (b) 3½ volumes phosphate buffer solution (pH 8), and (c) 9 volumes distilled water. Bioassay revealed the estrogenic potency of the outside fluids to be as follows: (a) 150 gamma estrone per cc., (b) 20 gamma estrone per cc., and (c) 20 gamma per cc. No precipitate was observed in either inside or outside fluids, a fact that is prticularly interesting in view of the frequent occurrence of precipitation following addition of water or buffer solution to estrone-20% dehydrocholate solutions. There is evidence that combination with bile acids increases the diffusibility of relatively poorly diffusible substances (Spanner and Bauman, 1932; Sobotka, 1937), although the reported data regarding their influence upon the diffusibility of cholesterol are contradictory (Breusch, 1937; Schoenheimer and Hrdina, 1931). The present findings suggest the presence of a rather firm, diffusible, water-soluble, estrone-dehydrocholate complex.

The fact that aqueous solutions of high estrogenic and androgenic potency can be prepared easily has obvious practical therapeutic implications, inasmuch as 5–10cc. of a 20% solution of sodium dehydrocholate can be given parenterally to human subjects with safety. The same is true of similar solutions of DCA and vitamin D and, also, of vitamin K and progesterone, if assays of the latter reveal that they retain their biological activity in such solutions. We have observed no untoward reaction after intravenous injection of 5 cc. of hormone-dehydrocholate solution in human subjects.

SUMMARY

A number of substances practically insoluble in water go into solution rather readily in aqueous solutions of sodium dehydrocholate. Among these are alpha-estradiol, estrone, estriol, progesterone, androsterone, testosterone, DCA, calciferol, diethylstilbestrol, methylstilbestrol, naphthalene and 2-methyl-1,4-naphthoquinone. In the case of estrone, diffusion of a water-soluble estrogenic substance through cellophane suggests the presence of a rather firm estrone-dehydrocholate complex. The possible nature of this phenomenon is discussed and its practical usefulness suggested.

A. Cantarow, K. E. Paschkis, A. E. Rakoff and L. P. Hansen

From the Jefferson Medical College and Hospital
Philadelphia, Pennsylvania

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EFFECT OF THIOUREA UPON FISH DEVELOPMENT[1]

THIOUREA and related substances have been shown to affect thyroid function in the amphibian (Gordon, Goldsmith and Charipper, 1943; Hughes and Astwood, 1944), chick (Mixner, Reineke and Turner, 1944; Astwood, Bissell and Hughes, 1944), rat (Mackenzie and Mackenzie, 1943; Astwood, Sullivan, Bissell, and Tyslowitz, 1943), rabbit (Baumann, Metzger and Marine, 1944) and the hyperthyroid human (Astwood, 1943; Williams and Bissell, 1943; Himsworth, 1943). Treatment with this drug is followed by an enlargement of the thyroid gland which is accompanied by a fall in the metabolic rate. These effects are ascribed to an interference by thiourea with the synthesis of normal thyroid hormone (Keston, Goldsmith, Gordon and Charipper, 1944; Franklin, Lerner and Chaikoff, 1944). In the young rat administration of thiouracil (Hughes, 1944), or thiourea (Goldsmith, Gordon and Charipper, 1944) results in a retardation of growth.

The feeding of mammalian desiccated thyroid powder has failed to influence either the rate of growth or the oxygen consumption of fishes (Drexler and v. Issekutz, 1935; Root and Etkin, 1937; Etkin, Root and Mofshin, 1940; Hasler and Meyer, 1942; Smith and Everett, 1943). The effects of excessive amounts of thyroid hormone upon sexual differentiation are controversial (Smith and Everett, 1943; Grobstein and Bellamy, 1939).

In view of these findings and since successful surgical thyroidectomy in fishes has not been reported it became of interest to ascertain whether the thyroid gland and development in fishes would be influenced by thiourea.

Three series of fishes were employed for these experiments.

They were of a hybrid strain involving *Platypoecilus maculatus* and *Xiphophorous hellerii*, New York Zoological Society culture #118. In this strain 2 phenotypes appear, one of which is a simple olive-green type and the other is a black spotted red variety. In the olive-green group, M. Gordon (unpublished data) found on the basis of over 300 adult fish that the ratio of males to females is 4 to 1. This closely approaches the results reported by Kosswig (1933), for apparently similar genetic strains.

In Series I seven immature (2 months old) olive-green fish were immersed in 4500 cc. of conditioned water containing 1.5 gm. of thiourea[2] (0.033%). A like number of immature animals served as untreated controls. Fresh solutions of conditioned water were made up biweekly. After 73 days of treatment a definite inhibition in the growth of thiourea treated animals was observed. An examination of the external sex characters as reflected in the structure of the anal fin and macroscopic study of the gonads, revealed that in the treated group of 7 fish only 1 showed a slight change towards maleness. The remaining 6 were sexually indifferent. Five of the 7 untreated control animals were definitely males and 2 were females. It would seem probable then that thiourea inhibits sexual maturation in these fishes.

In the second series 20 two week-old fish were treated similarly; 17 fish of the same brood served as controls. At intervals the fish were measured and some of them were fixed for histological examination; 79 days after treatment a final measurement was made.

In Series III 6 three month-old fish were subjected to cumulative doses

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[2] Eimer and Amend C. P.

of thiourea and 6 were untreated. The initial concentration was 0.033% and this was gradually increased to a final concentration of approximately 0.67%. Two of the treated animals died during the latter stages of exposure to the drug, and at the end of the experimental period (74 days) retarded growth was obvious in the remaining 4 fish.

The effects of thiourea upon the growth of 3 groups of fishes are summarized in Table 1.

The inhibition of growth in the thiourea-treated fishes is undoubtedly not due to inanition since throughout the experimental period these animals accepted the food as readily as did the untreated controls. Moreover, after

TABLE 1. EFFECT OF THIOUREA ON GROWTH OF FISH.

Series	No. of animals	No. days of treatment	Mean body length (mm.) ±avg. dev.
I Thiourea	7	73	22.7±3.8
Control	7	0	30.7±2.0
II Thiourea	12	79	11.3±1.1
Control	10	0	17.8±1.1
III Thiourea	4	74	35.0±2.5
Control	6	0	40.5±3.8

5 months of treatment the experimental animals are healthy, vigorous and show no signs of emaciation.

A histological examination of the thyroid glands of the thiourea-treated animals as compared with the controls shows a very definite hyperplasia. There is an easily apparent increase in the number of follicles visible in a single field. Evidence of extensive budding with indications of variations in size, shape and chromatin content of the nuclei is entirely in keeping with the hyperplastic nature of the tissue as a whole. The average individual follicle appears to be smaller and the dominant type of epithelium is low columnar as compared to the flattened epithelium seen invariably in the normal untreated fish. The colloid in the thyroids of the treated animals is scanty and the small amount which is present shows a decrease in density. Animals fixed after 49 days of treatment already presented these histological differences.

This work constitutes presumptive evidence that the thyroid gland is concerned with growth and the development of sex characters in fishes.

SUMMARY

Immersion of fishes in thiourea solutions results in an inhibition of growth and a failure in the development of the secondary sex characters. It appears that thiourea produces these effects by interfering with thyroid hormone production in much the same manner as reported for the mammal.

E. D. GOLDSMITH[3], ROSS F. NIGRELL,[4] ALBERT S. GORDON,
HARRY A. CHARIPPER AND MYRON GORDON[4]

From the Department of Biology, Washington Square College of Arts and Science,
New York University and the New York Zoological Society.

[3] Also of the College of the City of New York.
[4] New York Zoological Society.

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THE FAILURE OF CERTAIN STEROID HORMONES TO PREVENT ENLARGEMENT OF THE THYROID IN RATS FED THIOUREA

IT IS WELL established that adequate dosage of thiourea, thiouracil and related compounds is followed by a marked hyperplasia of the thyroid in the rat, accompanied by the signs and symptoms of hypothyroidism (Astwood, et al., 1943; MacKenzie and MacKenzie, 1943). The most tenable hypothesis to explain these findings holds that these compounds prevent the formation of the thyroid hormone; this lack of thyroid hormone results in hypothyroidism and removes the inhibiting effect of thyroid hormone on the release or production of hypophyseal thyrotrophic hormone; this in turn results in an excess production and release of hypophyseal thyrotrophic hormone which produces hyperplasia of thyroid (Astwood, et al., 1943; MacKenzie and MacKenzie, 1943).

It has been reported (Storck & Holcomb, 1942) that estrone decreases the basal metabolic rate in patients with hyperthyroidism sufficiently so that it has been employed in place of iodine before thyroidectomy with success in some patients. Also, estrogenic hormone administered to rats prevents or reverses those changes in the pituitary basophile cells which occur after thyroidectomy. (Nelson and Hickman, 1937; Leblond, Albert and Selye, 1942). Further, estrogens inhibit the production or release of the growth (Zondek, 1936), gonadotrophic (Zondek, 1936, Diaz, et al., 1938) and lactogenic (Nelson, 1936) complexes of the hypophysis. It is possible that the effect of estrone in hyperthyroidism occurs through inhibition of production or release of the hypophyseal thyrotrophic hormone. Therefore, studies were

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undertaken to determine whether administration of estrogens or androgens inhibits production or release of thyrotrophic hormone.

METHODS

Hyperplasia of the thyroid in rats fed thiourea was measured as an index of inhibition of thyrotrophic hormone production during administration of several different steroid hormones. Female rats of the inbred Fischer strain weighing 84 to 132 grams were used. In the place of water they were given a 1% thiourea solution in distilled water, ad libitum, and they were fed Rockland rat pellets. Hypophysectomy was performed on some of these animals by the usual parapharyngeal approach. The test animals received daily subcutaneous injections of the hormones in 0.1 ml. of sesame oil. Synthetic thyroxine, dissolved in a very dilute solution of sodium hydroxide, was injected subcutaneously daily in some rats.

After 21 days of treatment the rats were weighed and killed with ether. The thyroids were removed, weighed on a micro torsion balance and preserved in 10% formalin.

RESULTS

The results are summarized in Table 1. None of the compounds which were tested altered the weight of the thyroid in intact animals not fed thiourea. Thiourea alone produced a large increase in the weight of the thyroid. This was prevented by hypophysectomy as well as by 10 μg. of thyroxine daily. On the other hand, 50 μg. of estrone, 100 μg. of estrone, α-estradiol or diethylstilbestrol, or 0.5 mg. of testosterone propionate daily all failed to prevent the thyroid hyperplasia in rats receiving thiourea.

TABLE 1

Hormone treatment	No. of rats	1% thiourea	Initial wt. grams	Final wt. grams	Thyroid mg/100 gm.
None	4	0	101	118	7.6
None	5	+	111	98	33.8
Hypophysectomy	6	+	118	91	6.4
10μg. Thyroxine	7	0	99	113	6.8
10μg. Thyroxine	6	+	95	87	7.0
50μg. Estrone	4	0	101	126	7.6
50μg. Estrone	5	+	102	89	38.1
100μg. Estrone	5	0	112	132	7.1
100μg. Estrone	5	+	102	94	38.1
100μg.α-Estradiol	5	0	110	127	7.5
100μg.α-Estradiol	6	+	103	95	41.6
100μg. Diethylstilbestrol	6	0	117	110	7.4
100μg. Diethylstilbestrol	7	+	116	108	29.6
0.5 mg. Testosterone propionate	5	0	107	133	6.3
0.5 mg. Testosterone propionate	4	+	114	98	29.8

Microscopic examination of the thyroids of rats receiving thiourea alone revealed marked hyperplasia. The same degree of hyperplasia was also observed in those rats receiving both thiourea and a steroid hormone. Hypoplastic thyroids were found in animals which had undergone hypophysectomy, despite the administration of thiourea. The thyroids of the control animals, and the animals receiving both thiourea and thyroxine were essentially normal.

Some of the pituitaries were examined microscopically, but the short duration of the thiourea treatment precluded any conclusive findings.

DISCUSSION

These findings confirm the reported production of thyroid hyperplasia by administration of thiourea to rats and the prevention of this hyperplasia by thyroxine or by hypophysectomy.

Despite the fact that estrone, *a*-estradiol and diethylstilbestrol were administered in at least twice the dosage necessary to prevent the appearance of thyroidectomy cells in the pituitary (Leblond, et al., 1942), they failed to affect the thyroid hyperplasia in thiourea fed rats. Similarly, 0.5 mg. of testosterone propionate daily failed to prevent thyroid hyperplasia, although this compound fails to correct the thyroidectomy cells of the pituitary (Leblond, et al., 1942).

Thus, it appears that estrogens and androgens are not capable of inhibiting the excessive production and release of the hypophyseal thyrotrophic hormone which is believed to occur in rats fed thiourea.

SUMMARY

The hyperplasia of the thyroid in rats receiving thiourea is prevented by hypophysectomy and by administration of thyroxine, as others have reported.

Estrone, *a*-estradiol, diethylstilbestrol and testosterone propionate did not affect thyroid hyperplasia in rats fed thiourea.

The results are discussed in light of the reported effects of these compounds in thyroidectomized rats and in hyperthyroid patients.

ALBERT SEGALOFF[1,2]

From the Department of Anatomy, Wayne University, College of Medicine, Detroit, Michigan.

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[1] Ciba Fellow in Endocrinology.

[2] Present Address: Department of Medicine, Vanderbilt University, School of Medicine, Nashville 4, Tennessee.

NEW BOOKS

THE PERMEABILITY OF NATURAL MEMBRANES. By H. Davson, D.Sc., and J. F. Danielli, DSc., A.I.C. Cambridge Univ. Press and Macmillan Co., New York. $4.75. First edition, 1943. 361 pp. 73 figs.

The authors state that this book is intended as a general survey of the field of permeability, to assist those lecturing on the subject and to "accelerate the disappearence of the many errors which have crept into the literature designed for students." Included are materials of interest to students of zoology, botany, physiology, biochemistry and medicine.

In 21 chapters there is presented a fairly concise résumé of the classical and modern literature on the permeability of cells to various types of substances, hemolysis, effect of narcotics and temperature, structure of the plasma membrane, and the relation of permeability to secretion, kidney function, intestinal absorption, capillary permeability and so on. The book begins with a critique of methods of measuring permeability and of quantitative interpretation of the data and ends with a discussion of theories of permeability and the penetration of thin membranes, the last mentioned being in the form of an appendix by Danielli. The author of each chapter is indicated.

It is perhaps not generally realized that the study of permeability has passed through the initial descriptive phases and, chiefly through the work of the last 15 years, has entered upon a stage in which semi-quantitative experimentation is possible. This has led to a clarification of the theoretical aspects and, while it is not yet possible to formulate a satisfactory general theory of permeability, at least we can eliminate certain views which are no longer tenable (but which seem to survive indefinitely in text books). The authors are well equipped in point of experience and temperament to write this type of critical survey for they have contributed importantly during the last decade, chiefly in the quantitative and theoretical aspects.

The book does not, and was not meant to, cover all aspects of the field thoroughly. Thus, only five pages are devoted to permeability to gases and a similar amount to the effect of temperature on permeability. Only scattered references are given to the results obtained by the use of stable and radioactive tracers, though this method has proven quite powerful. Owing, no doubt, to difficulties of the present times, the literature beyond 1940, except for the work of the authors and their English colleagues, is not well covered. There are few typographical errors, the only one noticed by the reviewer being in Eq. 30, p. 213.

The casual reader may be misled by certain categorical statements which may or may not be modified in other sections of the book. Thus, on p. 23 and p. 30 it is stated that the erythrocyte membrane is permeable to anions but impermeable to cations. Later, (p. 140), the work of Cohn and

Cohn is referred to which shows that the dog erythrocyte is readily permeable to sodium. Still other isotope work might have been discussed which indicates that the traditional view of the specific cation permeability of tissue cells must be modified. It is doubtful that such results can be adequately explained on the basis of inhomogeneities in the membrane, or abnormal condition of the cells but rather involves metabolic and surface chemical processes, as suggested by Davson's own work and as referred to briefly in several places in the book.

The chapter on the relation of permeability and electrolyte pattern to bioelectric potentials is, in general, well written. However, more reliable data on nerve electrolytes and permeability are now available than those of Cowan, on which much of the discussion is based; the work of Steinbach is not mentioned.

Finally, the views of the authors, as stated in the last chapter and in the Appendix, offer a promising approach to a general theory of permeability and will doubtless help many to visualize a physical basis for the complex processes of permeability in terms of membrane ultrastructure, monolayer properties, electrokinetics, and so on. Exceptions may be taken to individual points in the argument and even to certain broad conclusions, but it represents the most coherent effort thus far attempted towards a reasonable interpretation of the facts.

In the opinion of the reviewer the book has successfully achieved the objectives set by the authors and will meet a long-felt need. It may be hoped that in later, happier years the authors will expand the book so as to deal more adequately with many subjects which were hardly more than mentioned in the present work.

F. O. Schmitt